BU
08/951,733
A-433B

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
C12N 5/16, 9/12, 15/12, 15/54, 15/62, 15/63, C07K 2/00, 14/47, 16/40, 16/18

A1

(11) International Publication Number: **WO 98/11204**

(43) International Publication Date: 19 March 1998 (19.03.98)

(21) International Application Number: PCT/US96/14679

(22) International Filing Date: 13 September 1996 (13.09.96)

(71) Applicant: GERON CORPORATION [US/US]; 200 Constitution Drive, Menlo Park, CA 94025 (US).

(72) Inventors: VILLEPONTEAU, Bryant; 1371 Greenbriar Road, San Carlos, CA 94070 (US). FENG, Junli; 1371 Greenbriar Road, San Carlos, CA 94070 (US). ANDREWS, William, H.; 6102 Park Avenue, Richmond, CA 94805 (US). ADAMS, Robert, R.; 419 Hillway Drive, Redwood City, CA 94062 (US).

(74) Agent: KASTER, Kevin, R.; Geron Corporation, 200 Constitution Drive, Menlo Park, CA 94025 (US).

(81) Designated States: AU, CA, JP, MX, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.

(54) Title: METHODS AND REAGENTS FOR REGULATING TELOMERE LENGTH AND TELOMERASE ACTIVITY

(57) Abstract

Purified and recombinant proteins TPC2 and TPC3 and recombinant or synthetic oligonucleotides corresponding to those proteins or fragments thereof can be used to detect regulators of telomere length and telomerase activity in mammalian cells and for a variety of related diagnostic and therapeutic purposes.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece			TR	Turkey
BG	Bulgaria	HU	Hungary	ML	Mali	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MN	Mongolia	UA	Ukraine
BR	Brazil	IL	Israel	MR	Mauritania	UG	Uganda
BY	Belarus	IS	Iceland	MW	Malawi	US	United States of America
CA	Canada	IT	Italy	MX	Mexico	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NE	Niger	VN	Viet Nam
CG	Congo	KE	Kenya	NL	Netherlands	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NO	Norway	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	NZ	New Zealand		
CM	Cameroon			PL	Poland		
CN	China	KR	Republic of Korea	PT	Portugal		
CU	Cuba	KZ	Kazakstan	RO	Romania		
CZ	Czech Republic	LC	Saint Lucia	RU	Russian Federation		
DE	Germany	LI	Liechtenstein	SD	Sudan		
DK	Denmark	LK	Sri Lanka	SE	Sweden		
EE	Estonia	LR	Liberia	SG	Singapore		

METHODS AND REAGENTS FOR REGULATING TELOMERE LENGTH AND TELOMERASE ACTIVITY

FIELD OF THE INVENTION

The present invention provides methods and reagents for regulating telomere length and modulating telomerase activity in mammalian cells as well as for detecting, diagnosing, and treating related diseases and conditions in humans and other mammals. In an important embodiment, the invention provides oligonucleotide probes and primers, polynucleotide plasmids, peptides, proteins, antibodies, and enzymes relating to genes and gene products that regulate telomere length and telomerase activity in mammalian cells. The invention has diverse applications and provides important advances in the fields of molecular biology, chemistry, pharmacology, and medical therapeutic and diagnostic technology.

BACKGROUND OF THE INVENTION

The DNA at the ends of the telomeres of chromosomes in mammalian cells consists of double- and single-stranded nucleic acid composed of many tandem repeats of a simple nucleotide sequence referred to as the telomeric repeat sequence. Telomeres help maintain chromosome structure and function; the loss of telomeric DNA can activate the cellular processes that detect and control DNA damage and monitor and control cell proliferation and senescence. The maintenance of telomeres and the regulation of telomere length are vital cellular functions involved in transmitting genetic information from generation to generation, aging, the control of cell growth, and cancer. See Harley, 1991, *Mutation Research* 256:271-282; and Blackburn, 1992, *Annu. Rev. Biochem.* 61:113-129, each of which is incorporated herein by reference (note: references cited herein are provided for convenience; such citations are not to be construed as an admission of prior invention).

The multi-component telomerase ribonucleoprotein enzyme catalyzes the synthesis of the first strand of telomeric DNA synthesized during telomere elongation, using the RNA component of the enzyme as a template. Although the RNA component of human telomerase (hTR) and other mammalian telomerase enzymes has been identified, isolated, characterized, and described in the scientific literature, the protein components of the telomerase enzyme as well as most other cellular macromolecules involved in telomere maintenance and the regulation of telomere length and telomerase activity in mammalian cells have

not. See Feng *et al.*, 1995, *Science* 269:1236-1241 and PCT patent publication No. 96/01835, each of which is incorporated herein by reference.

Many useful methods and reagents relating to telomere and telomerase biology have been described. See, e.g., U.S. Patent No. 5,489,508; PCT patent publication Nos. 95/23572, 95/13381, 95/13382, and 95/13383, each of which is incorporated herein by reference. Significant improvements to and new opportunities for telomere- and telomerase-mediated therapies as well as related assays, screens, diagnostic methods, and reagents could be realized and obtained, however, if additional cellular macromolecules involved in mammalian telomere maintenance and the regulation of telomere length and telomerase activity could be identified, characterized, and made available in pure or isolatable form. In particular, the characterization of the nucleotide and corresponding amino acid sequences of such macromolecules could provide new and useful recombinant expression vectors and plasmids, as well as related reagents useful in medical therapeutic and diagnostic technology.

SUMMARY OF THE INVENTION

The present invention provides methods and reagents for regulating telomere length and modulating telomerase activity in mammalian cells as well as for detecting, diagnosing, and treating related diseases and conditions in humans and other mammals.

In one embodiment, the invention provides recombinant mammalian host cells containing:

(i) a recombinant or synthetic nucleic acid comprising at least about 10 to 15 to 25 to 100 or more contiguous nucleotides corresponding to an open reading frame sequence of a human gene TPC2 contained in a human DNA insert of an ~3.5 kb *Not*I-*Bst*EII restriction fragment of plasmid pGRN109 (on deposit with the American Type Culture Collection under the accession number ATCC 97708); or

a synthetic or recombinant peptide or protein comprising at least about 6 to 10 to 15 to 25 to 100 or more contiguous amino acids corresponding to an amino acid sequence encoded by said open reading frame sequence; and

(ii) a recombinant or synthetic nucleic acid comprising at least about 10 to 15 to 25 to 100 or more contiguous nucleotides corresponding to an open reading frame sequence of a human gene TPC3 contained in a human DNA insert of an ~1.4 kb *Eco*RI-*Bam*HI restriction fragment of plasmid pGRN92 (ATCC 97707); or

a synthetic or recombinant peptide or protein comprising at least about 6 to 10 to 15 to 25 to 100 or more contiguous amino acids corresponding to an amino acid sequence encoded by said open reading frame sequence of gene TPC3;

said TPC2 and TPC3 genes characterized in coding for proteins that regulate telomere length or modulate telomerase activity and are present in human or other mammalian cells that express telomerase activity.

Other mammalian host cells provided by the invention include those that comprise either or both TPC2- and TPC3-derived recombinant or synthetic nucleic acids, peptides, or proteins. Furthermore, the invention also provides such cells further modified to contain a synthetic or recombinant nucleic acid comprising at least about 10 to 15 to 25 to 100 or more contiguous nucleotides corresponding to a contiguous nucleotide sequence of human hTR located in an ~2.5 kb *Hin*dIII-*Sac*I restriction fragment of pGRN33 (ATCC 75926).

The recombinant host cells of the invention have application in many useful methods also provided by the invention. For example, the invention provides recombinant host cells comprising novel expression vectors with expression control sequences operatively linked to nucleotide sequences encoding amino acids in a sequence substantially identical to the amino acid sequences encoded by the human TPC2 or TPC3 genes and, optionally, a recombinant hTR gene. These recombinant host cells are useful for producing recombinant human telomerase, for use in screens to identify agents that modulate telomerase activity or regulate telomere length, as well as for a variety of other purposes described more fully below. The recombinant host cells of the invention can also be incorporated into the germ line and/or somatic tissues of non-human transgenic mammals, as well as be administered to mammals for therapeutic purposes.

In another embodiment, the invention provides synthetic and recombinant oligonucleotides and nucleic acids in a variety of forms, i.e., isolatable, isolated, purified, or substantially pure, and for a variety of purposes, i.e., as probes or primers, as polynucleotide plasmids and vectors for introducing recombinant gene products that regulate telomere length or modulate telomerase activity in mammalian host cells, as restriction fragments for creating useful nucleic acids, and as reagents for therapeutic, diagnostic, and other applications. In particular, the invention provides recombinant or synthetic nucleic acids comprising at least about 10 to 15 to 25 to 100 or more contiguous nucleotides substantially identical or complementary in sequence to a contiguous nucleotide sequence located in either:

(i) an open reading frame sequence of a human gene TPC2 contained in a human DNA insert of an ~3.5 kb *Not*I-*Bst*EII restriction fragment of plasmid pGRN109; or

(ii) an open reading frame sequence of a human gene TPC3 contained in a human DNA insert of an ~1.4 kb *Eco*RI-*Bam*HI restriction fragment of plasmid pGRN92.

The novel oligonucleotide probes and primers of the invention typically comprise nucleotides in a sequence substantially identical or complementary to a sequence of nucleotides in a TPC2 or TPC3 gene or gene product to allow specific hybridization thereto in a complex mixture of nucleic acids. Such probes and primers therefore have useful application in a variety of diagnostic, therapeutic, and other applications.

The expression vectors of the invention typically comprise expression control sequences operatively linked to a nucleotide sequence encoding amino acids in a sequence identical to a sequence of amino acids in a TPC2 or TPC3 protein gene product. Such expression vectors have many useful applications, including in therapeutic methods of the invention as gene therapy vectors for modulating telomerase activity, either to activate or inhibit that activity, or for regulating telomere length, either to increase or decrease the length, in a target cell or tissue.

Gene therapy expression vectors of the invention also include those that encode variants or "muteins" of the TPC2 and/or TPC3 proteins, i.e., express proteins that differ from TPC2 and/or TPC3 by deletion, substitution, and/or addition of one or more amino acids. The gene therapy vectors of the invention may also, however, encode useful nucleic acids, such as hTR, or antisense nucleic acids or ribozymes that target the TPC2, TPC3, and/or hTR gene products, i.e., mRNA and telomerase RNA. Such vectors are useful in the therapeutic methods of the invention for treating or preventing diseases or conditions in which modulation of telomerase activity or telomere length can be of benefit. For example, in telomerase positive cancer cells, inhibition of telomerase activity can prevent telomere maintenance in those cells, inducing upon continued proliferation telomere loss, cell crisis, and death. For such purposes, the gene therapy vectors of the invention that express a non-functional TPC2 or TPC3 mutein or variant protein or other nucleic acid that can inhibit telomerase formation or telomere elongation by telomerase activity in the cell, such as by competing for RNA component or protein components, inhibition of endogenous gene expression, or other means, are preferred.

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
C12N 5/16, 9/12, 15/12, 15/54, 15/62, 15/63, C07K 2/00, 14/47, 16/40, 16/18

A1

(11) International Publication Number: WO 98/11204

(43) International Publication Date: 19 March 1998 (19.03.98)

(21) International Application Number: PCT/US96/14679

(22) International Filing Date: 13 September 1996 (13.09.96)

(71) Applicant: GERON CORPORATION [US/US]; 200 Constitution Drive, Menlo Park, CA 94025 (US).

(72) Inventors: VILLEPONTEAU, Bryant; 1371 Greenbriar Road, San Carlos, CA 94070 (US). FENG, Junli; 1371 Greenbriar Road, San Carlos, CA 94070 (US). ANDREWS, William, H.; 6102 Park Avenue, Richmond, CA 94805 (US). ADAMS, Robert, R.; 419 Hillway Drive, Redwood City, CA 94062 (US).

(74) Agent: KASTER, Kevin, R.; Geron Corporation, 200 Constitution Drive, Menlo Park, CA 94025 (US).

(81) Designated States: AU, CA, JP, MX, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.

(54) Title: METHODS AND REAGENTS FOR REGULATING TELOMERE LENGTH AND TELOMERASE ACTIVITY

(57) Abstract

Purified and recombinant proteins TPC2 and TPC3 and recombinant or synthetic oligonucleotides corresponding to those proteins or fragments thereof can be used to detect regulators of telomere length and telomerase activity in mammalian cells and for a variety of related diagnostic and therapeutic purposes.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece			TR	Turkey
BG	Bulgaria	HU	Hungary	ML	Mali	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MN	Mongolia	UA	Ukraine
BR	Brazil	IL	Israel	MR	Mauritania	UG	Uganda
BY	Belarus	IS	Iceland	MW	Malawi	US	United States of America
CA	Canada	IT	Italy	MX	Mexico	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NE	Niger	VN	Viet Nam
CG	Congo	KE	Kenya	NL	Netherlands	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NO	Norway	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	NZ	New Zealand		
CM	Cameroon			PL	Poland		
CN	China	KR	Republic of Korea	PT	Portugal		
CU	Cuba	KZ	Kazakstan	RO	Romania		
CZ	Czech Republic	LC	Saint Lucia	RU	Russian Federation		
DE	Germany	LI	Liechtenstein	SD	Sudan		
DK	Denmark	LK	Sri Lanka	SE	Sweden		
EE	Estonia	LR	Liberia	SG	Singapore		

METHODS AND REAGENTS FOR REGULATING TELOMERE LENGTH AND TELOMERASE ACTIVITY

FIELD OF THE INVENTION

The present invention provides methods and reagents for regulating telomere length and modulating telomerase activity in mammalian cells as well as for detecting, diagnosing, and treating related diseases and conditions in humans and other mammals. In an important embodiment, the invention provides oligonucleotide probes and primers, polynucleotide plasmids, peptides, proteins, antibodies, and enzymes relating to genes and gene products that regulate telomere length and telomerase activity in mammalian cells. The invention has diverse applications and provides important advances in the fields of molecular biology, chemistry, pharmacology, and medical therapeutic and diagnostic technology.

BACKGROUND OF THE INVENTION

The DNA at the ends of the telomeres of chromosomes in mammalian cells consists of double- and single-stranded nucleic acid composed of many tandem repeats of a simple nucleotide sequence referred to as the telomeric repeat sequence. Telomeres help maintain chromosome structure and function; the loss of telomeric DNA can activate the cellular processes that detect and control DNA damage and monitor and control cell proliferation and senescence. The maintenance of telomeres and the regulation of telomere length are vital cellular functions involved in transmitting genetic information from generation to generation, aging, the control of cell growth, and cancer. See Harley, 1991, *Mutation Research* 256:271-282; and Blackburn, 1992, *Annu. Rev. Biochem.* 61:113-129, each of which is incorporated herein by reference (note: references cited herein are provided for convenience; such citations are not to be construed as an admission of prior invention).

The multi-component telomerase ribonucleoprotein enzyme catalyzes the synthesis of the first strand of telomeric DNA synthesized during telomere elongation, using the RNA component of the enzyme as a template. Although the RNA component of human telomerase (hTR) and other mammalian telomerase enzymes has been identified, isolated, characterized, and described in the scientific literature, the protein components of the telomerase enzyme as well as most other cellular macromolecules involved in telomere maintenance and the regulation of telomere length and telomerase activity in mammalian cells have

not. See Feng *et al.*, 1995, *Science* 269:1236-1241 and PCT patent publication No. 96/01835, each of which is incorporated herein by reference.

Many useful methods and reagents relating to telomere and telomerase biology have been described. See, e.g., U.S. Patent No. 5,489,508; PCT patent publication Nos. 95/23572, 95/13381, 95/13382, and 95/13383, each of which is incorporated herein by reference. Significant improvements to and new opportunities for telomere- and telomerase-mediated therapies as well as related assays, screens, diagnostic methods, and reagents could be realized and obtained, however, if additional cellular macromolecules involved in mammalian telomere maintenance and the regulation of telomere length and telomerase activity could be identified, characterized, and made available in pure or isolatable form. In particular, the characterization of the nucleotide and corresponding amino acid sequences of such macromolecules could provide new and useful recombinant expression vectors and plasmids, as well as related reagents useful in medical therapeutic and diagnostic technology.

SUMMARY OF THE INVENTION

The present invention provides methods and reagents for regulating telomere length and modulating telomerase activity in mammalian cells as well as for detecting, diagnosing, and treating related diseases and conditions in humans and other mammals.

In one embodiment, the invention provides recombinant mammalian host cells containing:

(i) a recombinant or synthetic nucleic acid comprising at least about 10 to 15 to 25 to 100 or more contiguous nucleotides corresponding to an open reading frame sequence of a human gene TPC2 contained in a human DNA insert of an ~3.5 kb *Not*I-*Bst*EII restriction fragment of plasmid pGRN109 (on deposit with the American Type Culture Collection under the accession number ATCC 97708); or

a synthetic or recombinant peptide or protein comprising at least about 6 to 10 to 15 to 25 to 100 or more contiguous amino acids corresponding to an amino acid sequence encoded by said open reading frame sequence; and

(ii) a recombinant or synthetic nucleic acid comprising at least about 10 to 15 to 25 to 100 or more contiguous nucleotides corresponding to an open reading frame sequence of a human gene TPC3 contained in a human DNA insert of an ~1.4 kb *Eco*RI-*Bam*HI restriction fragment of plasmid pGRN92 (ATCC 97707); or

a synthetic or recombinant peptide or protein comprising at least about 6 to 10 to 15 to 25 to 100 or more contiguous amino acids corresponding to an amino acid sequence encoded by said open reading frame sequence of gene TPC3;

said TPC2 and TPC3 genes characterized in coding for proteins that regulate telomere length or modulate telomerase activity and are present in human or other mammalian cells that express telomerase activity.

Other mammalian host cells provided by the invention include those that comprise either or both TPC2- and TPC3-derived recombinant or synthetic nucleic acids, peptides, or proteins. Furthermore, the invention also provides such cells further modified to contain a synthetic or recombinant nucleic acid comprising at least about 10 to 15 to 25 to 100 or more contiguous nucleotides corresponding to a contiguous nucleotide sequence of human hTR located in an ~2.5 kb *Hin*dIII-*Sac*I restriction fragment of pGRN33 (ATCC 75926).

The recombinant host cells of the invention have application in many useful methods also provided by the invention. For example, the invention provides recombinant host cells comprising novel expression vectors with expression control sequences operatively linked to nucleotide sequences encoding amino acids in a sequence substantially identical to the amino acid sequences encoded by the human TPC2 or TPC3 genes and, optionally, a recombinant hTR gene. These recombinant host cells are useful for producing recombinant human telomerase, for use in screens to identify agents that modulate telomerase activity or regulate telomere length, as well as for a variety of other purposes described more fully below. The recombinant host cells of the invention can also be incorporated into the germ line and/or somatic tissues of non-human transgenic mammals, as well as be administered to mammals for therapeutic purposes.

In another embodiment, the invention provides synthetic and recombinant oligonucleotides and nucleic acids in a variety of forms, i.e., isolatable, isolated, purified, or substantially pure, and for a variety of purposes, i.e., as probes or primers, as polynucleotide plasmids and vectors for introducing recombinant gene products that regulate telomere length or modulate telomerase activity in mammalian host cells, as restriction fragments for creating useful nucleic acids, and as reagents for therapeutic, diagnostic, and other applications. In particular, the invention provides recombinant or synthetic nucleic acids comprising at least about 10 to 15 to 25 to 100 or more contiguous nucleotides substantially identical or complementary in sequence to a contiguous nucleotide sequence located in either:

(i) an open reading frame sequence of a human gene TPC2 contained in a human DNA insert of an ~3.5 kb *Not*I-*Bst*EII restriction fragment of plasmid pGRN109; or

(ii) an open reading frame sequence of a human gene TPC3 contained in a human DNA insert of an ~1.4 kb *Eco*RI-*Bam*HI restriction fragment of plasmid pGRN92.

The novel oligonucleotide probes and primers of the invention typically comprise nucleotides in a sequence substantially identical or complementary to a sequence of nucleotides in a TPC2 or TPC3 gene or gene product to allow specific hybridization thereto in a complex mixture of nucleic acids. Such probes and primers therefore have useful application in a variety of diagnostic, therapeutic, and other applications.

The expression vectors of the invention typically comprise expression control sequences operatively linked to a nucleotide sequence encoding amino acids in a sequence identical to a sequence of amino acids in a TPC2 or TPC3 protein gene product. Such expression vectors have many useful applications, including in therapeutic methods of the invention as gene therapy vectors for modulating telomerase activity, either to activate or inhibit that activity, or for regulating telomere length, either to increase or decrease the length, in a target cell or tissue.

Gene therapy expression vectors of the invention also include those that encode variants or "muteins" of the TPC2 and/or TPC3 proteins, i.e., express proteins that differ from TPC2 and/or TPC3 by deletion, substitution, and/or addition of one or more amino acids. The gene therapy vectors of the invention may also, however, encode useful nucleic acids, such as hTR, or antisense nucleic acids or ribozymes that target the TPC2, TPC3, and/or hTR gene products, i.e., mRNA and telomerase RNA. Such vectors are useful in the therapeutic methods of the invention for treating or preventing diseases or conditions in which modulation of telomerase activity or telomere length can be of benefit. For example, in telomerase positive cancer cells, inhibition of telomerase activity can prevent telomere maintenance in those cells, inducing upon continued proliferation telomere loss, cell crisis, and death. For such purposes, the gene therapy vectors of the invention that express a non-functional TPC2 or TPC3 mutein or variant protein or other nucleic acid that can inhibit telomerase formation or telomere elongation by telomerase activity in the cell, such as by competing for RNA component or protein components, inhibition of endogenous gene expression, or other means, are preferred.

In another embodiment, the present invention provides peptides, proteins, antibodies, and enzymes, relating to genes and gene products that regulate telomere length and telomerase activity in mammalian cells. In particular, the invention provides synthetic or recombinant peptides or proteins comprising at least about 6 to 10 to 15 to 25 to 100 or more contiguous amino acids identical in sequence to an amino acid sequence encoded by an open reading frame sequence of a human gene located in either:

(i) an ~3.5 kb *Not*I-*Bst*EII restriction fragment of plasmid pGRN109; or

(ii) an ~1.4 kb *Eco*RI-*Bam*HI restriction fragment of plasmid pGRN92.

The present invention provides the proteins encoded by the TPC2 and TPC3 genes in isolatable form from host cells expressing recombinant TPC2 and/or TPC3 protein, as well as in purified and substantially pure form from synthesis *in vitro* or by purification from recombinant host cells or by purification of the naturally occurring proteins using antibodies or other reagents of the invention. Such proteins have application in methods for reconstituting *in vitro* telomerase or other enzymatic activities that maintain telomeres and regulate telomere length. These methods in turn have application in screens for therapeutic agents, for diagnostic tests, and for other applications. In addition, peptides corresponding to the amino acid sequences of TPC2 or TPC3 proteins can also be used to regulate telomere length and telomerase activity in mammalian cells.

The proteins and peptides of the invention can also be used to generate antibodies specific for TPC2 or TPC3 proteins or for particular epitopes on those proteins. Thus the invention provides polyclonal and monoclonal antibodies that specifically bind to TPC2 or TPC3 proteins. These antibodies can in turn be used to isolate TPC2 or TPC3 proteins from normal or recombinant cells and so can be used to purify the proteins as well as other proteins associated therewith. These antibodies also have important application in the detection of cells comprising TPC2 or TPC3 proteins in complex mixtures of cells. Such detection methods have application in screening, diagnosing, and monitoring diseases and other conditions, such as cancer, pregnancy, or fertility, because the TPC2 and TPC3 proteins are present in most cells capable of elongating telomeric DNA and expressing telomerase activity.

The immunogenic peptides and proteins of the invention can also be used in therapeutic immunization and vaccination procedures. The invention provides a method of immunizing a subject, as well as vaccines useful in the method, against cells that maintain telomeres and express telomerase activity that comprises administering an immunostimulating amount of such peptides or proteins of the invention.

In another embodiment, the invention provides a subtraction hybridization differential display method to identify, isolate, and clone expressed sequence tags (ESTs) of mRNA species encoding rare proteins, such as those involved in telomere elongation and the regulation of telomere length and telomerase activity. This method comprises the steps of:

(i) obtaining mRNA from a first population of mammalian cells which contain said rare protein, i.e., a protein component of telomerase, and from a second population of mammalian cells which do not contain said rare protein;

(ii) subjecting such mRNA to reverse-transcription and second-strand synthesis to form first and second cDNA preparations, said first and second cDNA preparations differing from one another with respect to presence or absence of cDNA molecules encoding said rare protein and a label incorporated into one of said first and second cDNA preparations;

(iii) combining said cDNA preparations under conditions such that complementary strands of cDNA from said first and second cDNA preparations anneal to form a mixture of double-stranded and single-stranded cDNA; and

(iv) separating cDNA comprising said label from cDNA that does not, thereby forming an isolated preparation of cDNA from said first population that has been depleted from complementary cDNA in said second population and enriched for said cDNA encoding said rare protein.

Steps (iii) and (iv) of the above method can be repeated as often as desired, and the cDNA isolated after completion of step (iv) can be amplified by PCR, to provide cDNA preparations greatly enriched for the desired cDNA.

These and other embodiments of the invention will be described in detail below.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1, in parts A, B, and C, is a bar graph showing the results of RT-PCR analysis using primers specific for TPC2 (Figure 1A) or TPC3 (Figure 1B) cDNA. In this and the other bar graphs, the number over each bar is the numerical result obtained; for RT-PCR results, this number was generated by scanning autoradiograms or PhosphorImager™ screens (Molecular Dynamics) of the RT-PCR products after gel electrophoresis. Under these test conditions, TPC2 and TPC3 mRNA is absent or detectable only at very low levels in the telomerase negative cell lines tested (labeled "Mortal" in the Figure) and detectable in all (most at clearly detectable levels) telomerase positive cell lines tested (labeled "Immortal" in the Figure). Figure 1C shows TPC3 mRNA levels normalized to GAPDH levels and illustrates the difference in TPC3 mRNA levels between mortal and immortal cells (the spaces marked "0.0" are provided merely as breaks

in the graphed data). GAPDH mRNA was used as a control; due to its greater abundance, the RT-PCR of the GAPDH samples was allowed to complete fewer cycles of PCR than used for the TPC2 or TPC3 samples.

Figure 2, in parts A, B, and C, is a bar graph showing the results of an RT-PCR analysis of hTR RNA and TPC2 and TPC3 mRNA levels as well as telomerase activity in a variety of cell lines. Figure 2A shows TPC2 and TPC3 mRNA levels normalized to GAPDH mRNA levels in various cell lines, all of which are telomerase positive except IMR-90, and demonstrates a correlation in the levels of these two telomere length and telomerase activity regulatory proteins. Figure 2B shows how TPC3 mRNA levels correlate with telomerase activity (as measured using the TRAP assay) in a variety of cell lines. The IMR90, HTB-153, WI-38 VA13, KMSF, and T0 (unactivated T cells; note that T7 represents activated T cells) express no or only very low levels of telomerase activity. Figure 2C shows how hTR RNA levels correlate with telomerase activity levels in a variety of cell lines. Taken together, these results show that TPC2 and TPC3 mRNA levels correlate with hTR levels and with telomerase activity levels in a variety of mortal and immortal cells lines.

Figure 3 shows a restriction site and function map of the ~7.2 kb plasmid pGRN109, which contains an ~3.5 kb *Not*I-*Bst*EII restriction fragment that contains an ~3.3 kb open reading frame encoding the TPC2 protein (labeled "ORF" and "TPC2").

Figure 4 lists portions of the nucleotide sequence and deduced amino acid sequence of the TPC2 open reading frame corresponding to the human TPC2 gene, mRNA, and protein products. In the Figure, as well as throughout the specification and Figures, nucleotides and amino acids are represented using standard abbreviations and designations; however, ambiguous nucleotides are represented as shown in the key at the bottom of Figure 4. The initiating methionine codon is believed to be at nucleotides 16-18 of the sequence; the termination codon is marked with "---".

Figure 5 shows a restriction site and function map of the ~8 kb plasmid pGRN92, which contains an ~1.4 kb *Eco*RI-*Bam*HI restriction fragment that contains an ~1.1 kb open reading frame encoding the TPC3 protein (labeled "ORF" and "TPC3").

Figure 6 lists the nucleotide sequence and deduced amino acid sequence of the TPC3 open reading frame corresponding to the human TPC3 gene, mRNA, and protein products. The initiating methionine codon is marked with "***" and the stop codon with "---".

Figure 7 shows the results of an analysis of telomerase activity levels in stable recombinant HeTe7 clones expressing the sense or antisense mRNA of gene TPC3 or a control vector. The recombinant sense TPC3 mRNA reduced telomerase activity markedly in these cells.

Figure 8 shows the results of an analysis of telomere length in stable recombinant HeTe7 clones expressing the sense or antisense mRNA of gene TPC3 or a control vector. The recombinant TPC3 sense mRNA decreased telomere length (mean TRF) in the cells.

Figure 9 lists the nucleotide sequence of the hTR gene and corresponding RNA transcript; the sequence shown is that of one strand of an ~1 kb *PstI* restriction fragment that can be isolated from plasmid pGRN33. The sequence of the mature hTR transcript, which serves as the template in the telomerase ribonucleoprotein, is marked with asterisks -- the 3' end of the transcript is marked with an ">".

These Figures are discussed in more detail below, where a variety of preferred embodiments of the invention are described.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention provides methods and reagents for regulating telomere length and modulating telomerase activity in mammalian cells as well as for detecting, diagnosing, and treating related diseases and conditions in humans and other mammals. To facilitate understanding and practice of the invention in its many and diverse applications, this description is organized as shown below.

I. DEFINITIONS

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by those of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the

present invention, preferred methods and materials are described. For purposes of the present invention, the following terms are defined below.

"Antibody" refers to naturally occurring and recombinant polypeptides and proteins encoded by immunoglobulin genes, or fragments thereof, that specifically bind to or "recognize" an analyte or "antigen". Immunoglobulin genes include the kappa, lambda, alpha, gamma, delta, epsilon and mu constant region genes, as well as myriad immunoglobulin variable region genes. An antibody can exist as an intact immunoglobulin or as any one of a number of well characterized fragments, e.g., Fab' and $F(ab)'_2$ fragments, produced by various means, including recombinant methodology and digestion with various peptidases.

"cDNA" refers to deoxyribonucleic acids produced by reverse-transcription and typically second-strand synthesis of mRNA or other RNA produced by a gene; if double-stranded, a cDNA molecule has both a coding or sense and a non-coding or antisense strand.

"Complementary to" refers to a polynucleotide sequence that can hybridize specifically to another polynucleotide sequence; for example, a nucleic acid comprising nucleotides in the sequence "5'-TATAC" is complementary to a nucleic acid comprising nucleotides in the sequence "5'-GTATA".

"Corresponds to" or "corresponding to" refers to (i) a polynucleotide having a nucleotide sequence that is substantially identical or complementary to all or a portion of a reference polynucleotide sequence or encoding an amino acid sequence identical to an amino acid sequence in a peptide or protein; or (ii) a peptide or polypeptide having an amino acid sequence that is substantially identical to a sequence of amino acids in a reference peptide or protein.

"Encoding" refers to the inherent property of specific sequences of nucleotides in a nucleic acid, such as a gene in a chromosome or an mRNA, to serve as templates for synthesis of other polymers and macromolecules in biological processes having a defined sequence of nucleotides (i.e., rRNA, tRNA, other RNA molecules) or amino acids and the biological properties resulting therefrom. Thus a gene encodes a protein, if transcription and translation of mRNA produced by that gene produces the protein in a cell or other biological system. Both the coding strand, the nucleotide sequence of which is identical to the mRNA sequence and is usually provided in sequence listings, and non-coding strand, used as the template for transcription, of a gene or cDNA can be referred to as encoding the protein or other product of that gene or cDNA. A nucleic acid that encodes a protein includes any nucleic acids that have different nucleotide sequences but encode the same amino acid sequence of the protein due to the

degeneracy of the genetic code. Nucleic acids and nucleotide sequences that encode proteins may include introns.

"Expression control sequence" refers to nucleotide sequences in nucleic acids that regulate the expression (transcription and/or translation) of a nucleotide sequence operatively linked thereto. Expression control sequences can include, for example and without limitation, sequences of promoters, enhancers, transcription terminators, a start codon (i.e., ATG), splicing signals for introns, and stop codons.

"Immunoassay" refers to an assay that utilizes an antibody to bind an analyte specifically. An immunoassay is characterized by the use of specific binding properties of a particular antibody to isolate, target, and/or quantify the amount of an analyte.

"Label" or "labeled" refers to a detectable marker and to the incorporation of such a marker into a nucleic acid, protein, or other molecule. The label may be detectable directly, i.e., the label can be a radioisotope (e.g., ^{3}H, ^{14}C, ^{35}S, ^{125}I, ^{131}I) or a fluorescent or phosphorescent molecule (e.g., FITC, rhodamine, lanthanide phosphors), or indirectly, i.e., by enzymatic activity (e.g., horseradish peroxidase, beta-galactosidase, luciferase, alkaline phosphatase) or ability to bind to another molecule (e.g., streptavidin, biotin, an epitope). Incorporation of a label can be achieved by a variety of means, i.e., by use of radiolabeled or biotinylated nucleotides in polymerase-mediated primer extension reactions, epitope-tagging, or binding to an antibody. Labels can be attached directly or via spacer arms of various lengths to reduce steric hindrance.

"Naturally occurring" refers to a substance, typically an amino acid, nucleotide, nucleic acid, or protein, that exists in nature without human intervention. For example, deoxyribonucleic acid or DNA is naturally occurring.

"Oligonucleotide" refers to a polymer composed of a multiplicity of nucleotide units (ribonucleotides or deoxyribonucleotides or related structural variants or synthetic analogs thereof) linked via phosphodiester bonds (or related structural variants or synthetic analogs thereof). Thus, while the term "oligonucleotide" typically refers to a nucleotide polymer in which the nucleotides and the linkages between them are naturally occurring; the term also refers to various analogs, such as, for example and without limitation, peptide-nucleic acids (PNAs), phosphoramidates, phosphorothioates, methyl phosphonates, 2-O-methyl ribonucleic acids, and the like. An oligonucleotide is typically rather short in length, generally from about 10 to 30 nucleotides, but the term can refer to molecules of any length, although the term "polynucleotide" or "nucleic acid" is typically used for large oligonucleotides.

"Open reading frame" refers to a nucleotide sequence that encodes a polypeptide or protein and is bordered on the 5'-end by an initiation codon (ATG) or another codon that does not encode a stop codon and on the 3'-end by a stop codon but otherwise does not contain any in-frame stop codons between the codons at the 5'-border and the 3'-border.

"Pharmaceutical composition" refers to a composition suitable for pharmaceutical use in a mammal. A pharmaceutical composition comprises a pharmacologically effective amount of an active agent and a pharmaceutically acceptable carrier. "Pharmacologically effective amount" refers to that amount of an agent effective to produce the intended pharmacological result. "Pharmaceutically acceptable carrier" refers to any of the standard pharmaceutical carriers, buffers, and excipients, such as a phosphate buffered saline solution, 5% aqueous solution of dextrose, and emulsions, such as an oil/water or water/oil emulsion, and various types of wetting agents and/or adjuvants. Suitable pharmaceutical carriers and formulations are described in *Remington's Pharmaceutical Sciences*, 19th Ed. (Mack Publishing Co., Easton, 1995). Preferred pharmaceutical carriers depend upon the intended mode of administration of the active agent. Typical modes of administration include enteral (i.e., oral) or parenteral (i.e., subcutaneous, intramuscular, or intravenous intraperitoneal injection; or topical, transdermal, or transmucosal administration).

"Physiological conditions" refer to temperature, pH, ionic strength, viscosity, and like biochemical parameters that are compatible with a viable organism and/or that typically exist intracellularly in a viable mammalian cell. For example, the intracellular conditions in a mammalian cell grown under typical laboratory culture conditions are physiological conditions. Suitable *in vitro* reaction conditions for PCR and many polynucleotide enzymatic reactions and manipulations are generally physiological conditions. In general, *in vitro* physiological conditions comprise 50-200 mM NaCl or KCl, pH 6.5-8.5, 20-45 degrees C, and 0.001-10 mM divalent cation (e.g., Mg^{++}, Ca^{++}); preferably about 150 mM NaCl or KCl, pH 7.2-7.6, 5 mM divalent cation, and, often, including 0.01-1.0 percent nonspecific protein (e.g., BSA). A non-ionic detergent (Tween, NP-40, Triton X-100) can also be present, usually at about 0.001 to 2%, typically 0.05-0.2% (v/v). Particular aqueous conditions may be selected by the practitioner according to conventional methods. For general guidance, the following buffered aqueous conditions may be applicable: 10-250 mM NaCl, 5-50 mM Tris HCl, pH 5-8, with optional addition of divalent cation(s) and/or metal chelators and/or nonionic detergents and/or membrane fractions and/or antifoam agents and/or scintillants.

"Polynucleotide" or "nucleic acid" refers to an oligonucleotide and is typically used to refer to oligonucleotides greater than 30 nucleotides in length. Conventional notation is used herein to portray polynucleotide sequences: the left-hand end of single-stranded polynucleotide sequences is the 5'-end; the left-hand direction of double-stranded polynucleotide sequences is referred to as the 5'-direction. The direction of 5' to 3' addition of nucleotides to nascent RNA transcripts is referred to as the transcription direction; the DNA strand having the same sequence as an mRNA is referred to as the "coding strand"; sequences on the DNA strand having the same sequence as an mRNA transcribed from that DNA and which are located 5' to the 5'-end of the RNA transcript are referred to as "upstream sequences"; sequences on the DNA strand having the same sequence as the RNA and which are 3' to the 3' end of the coding RNA transcript are referred to as "downstream sequences". Polynucleotides and recombinantly produced protein, and fragments or analogs thereof, may be prepared according to methods known in the art and described in Maniatis *et al., Molecular Cloning: A Laboratory Manual*, 2nd Ed., (1989), Cold Spring Harbor, N.Y., and Berger and Kimmel, *Methods in Enzymology, Volume 152, Guide to Molecular Cloning Techniques* (1987), Academic Press, Inc., San Diego, CA, which are incorporated herein by reference.

"Polypeptide," "peptide" and "protein" are used interchangeably herein to refer to a polymer of amino acid residues and to variants and synthetic analogs of the same. Thus, these terms apply to amino acid polymers in which one or more amino acid residues is a synthetic non-naturally occurring amino acid, such as a chemical analog of a corresponding naturally occurring amino acid, as well as to naturally occurring amino acid polymers. Conventional notation is used herein to portray polypeptide sequences: the left-hand end of polypeptide sequences is the amino-terminus; the right-hand end of polypeptide sequences is the carboxy-terminus. The term "recombinant protein" refers to a protein that is produced by expression of a recombinant DNA molecule that encodes the amino acid sequence of the protein. Terms used to describe sequence relationships between two or more polynucleotides or polypeptides include "reference sequence", "comparison window", "sequence identity", "percentage of sequence identity", and "substantial identity". A "reference sequence" is a defined sequence used as a basis for a sequence comparison and may be a subset of a larger sequence, i.e., a complete cDNA, protein, or gene sequence. Generally, a reference sequence is at least 12 but frequently 15 to 18 and often at least 25 nucleotides (or other monomer unit) in length. Because two polynucleotides may each comprise (1) a sequence (i.e., only a portion of the complete polynucleotide sequence) that is

similar between the two polynucleotides, and (2) a sequence that is divergent between the two polynucleotides, sequence comparisons between two (or more) polynucleotides are typically performed by comparing sequences of the two polynucleotides over a "comparison window" to identify and compare local regions of sequence similarity. A "comparison window" refers to a conceptual segment of typically at least 12 contiguous residues that is compared to a reference sequence; the comparison window may comprise additions or deletions (i.e., gaps) of about 20 percent or less as compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences. Optimal alignment of sequences for aligning a comparison window may be conducted by computerized implementations of algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package Release 7.0, Genetics Computer Group, 575 Science Dr., Madison, WI) or by inspection, and the best alignment (i.e., resulting in the highest percentage of homology over the comparison window) generated by any of the various methods is selected.

"Primer" refers to an oligonucleotide, i.e., a purified restriction fragment or a synthetic oligonucleotide, that is capable of acting as a point of initiation of synthesis when placed under conditions in which synthesis of a primer extension product complementary to a nucleic acid strand (the "template") is induced, i.e., in the presence of nucleotides and an agent for polymerization such as DNA polymerase and at a suitable temperature and pH. The primer is preferably single-stranded for maximum efficiency in amplification but may alternatively be double-stranded. If double stranded, the primer may need to be treated to separate its strands before being used to prepare extension products. Primers are typically oligodeoxyribonucleotides, but a wide variety of synthetic and non-naturally occurring oligonucleotide primers can be used for various applications. A primer must be sufficiently long to prime the synthesis of extension products in the presence of the agent for polymerization. The length of a primer depends on many factors, including application, temperature to be employed, template, reaction conditions, other reagents, and source of primers. For example, depending on the complexity of the target sequence, the oligonucleotide primer typically contains 15-25 or more nucleotides, although it may contain fewer nucleotides. Short primer molecules generally require cooler temperatures to form stable hybrid complexes with template. Primers can be large polynucleotides, such as from about 200 nucleotides to several kilobases or more. A primer must be substantially complementary to the sequence on the template to which it is designed to hybridize to serve as a site for the initiation of synthesis

but need not reflect the exact sequence of the template. For example, non-complementary nucleotides may be attached to the 5'-end of the primer, with the remainder of the primer sequence being complementary to the template. Alternatively, non-complementary nucleotides or longer sequences can be interspersed into a primer, provided that the primer sequence has sufficient complementarity with the sequence of the template to hybridize therewith and thereby form a template for synthesis of the extension product of the primer.

"Probe" refers to a molecule that binds to a specific sequence or sub-sequence or other moiety of another molecule. Unless otherwise indicated, the term "probe" typically refers to an oligonucleotide probe that binds to another nucleic acid, often called the "target nucleic acid", through complementary base pairing. Probes may bind target nucleic acids lacking complete sequence complementarity with the probe, depending upon the stringency of the hybridization conditions. Probes can be directly or indirectly labeled.

"Recombinant" refers to methods and reagents in which nucleic acids synthesized or otherwise manipulated *in vitro* are used to produce gene products encoded by those nucleic acids in cells or other biological systems. For example, an amplified or assembled product polynucleotide may be inserted into a suitable DNA vector, such as a bacterial plasmid, and the plasmid can be used to transform a suitable host cell. The gene is then expressed in the host cell to produce the recombinant protein. The transformed host cell may be prokaryotic or eukaryotic, including bacterial, mammalian, yeast, *Aspergillus*, and insect cells. A recombinant polynucleotide may serve a non-coding function (e.g., promoter, origin of replication, ribosome-binding site, etc.) as well.

"Recombinant host cell" refers to a cell that comprises a recombinant nucleic acid molecule, typically a recombinant plasmid or other expression vector. Thus, for example, recombinant host cells can express genes that are not found within the native (non-recombinant) form of the cell.

"Selected from" refers, in connection with sequences, to one sequence sharing identity with another sequence.

"Sequence identity" refers to sequences that are identical (i.e., on a nucleotide-by-nucleotide or amino acid-by-amino acid basis) over the window of comparison. The term "percentage of sequence identity" is calculated by comparing two optimally aligned sequences over the window of comparison, determining the number of positions at which the identical nucleic acid base (e.g., A, T, C, G, U, or I) occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of

positions in the window of comparison (i.e., the window size), and multiplying the result by 100 to yield the percentage of sequence identity.

"Specifically binds to" refers to the ability of one molecule, typically a macromolecule such as an antibody or oligonucleotide, to contact and associate with another specific molecule even in the presence of many other diverse molecules. For example, a single-stranded nucleic acid can "specifically bind to" a single-stranded oligonucleotide that is complementary in sequence, and an antibody "specifically binds to" or "is specifically immunoreactive with" its corresponding antigen. Thus, under designated immunoassay conditions, an antibody binds preferentially to a particular protein and not in a significant amount to other proteins present in the sample. Specific binding to a protein under such conditions requires an antibody selected for its specificity for a particular protein. To select antibodies specifically immunoreactive with a particular protein, one can employ a variety of means, i.e., solid-phase ELISA immunoassays are routinely used to select monoclonal antibodies specifically immunoreactive with a protein. See Harlow and Lane (1988), *Antibodies, A Laboratory Manual*, Cold Spring Harbor Publications, New York.

"Specific hybridization" refers to the formation of hybrids between a probe polynucleotide (e.g., a polynucleotide of the invention which may include substitutions, deletions, and/or additions) and a specific target polynucleotide (e.g., a polynucleotide having the sequence of a TPC2 or TPC3 gene or gene product), wherein the probe preferentially hybridizes to the specific target and not to other polynucleotides in the mixture that do not share sequence identity with the target.

"Substantial identity" or "substantially identical" denotes a characteristic of a polynucleotide or polypeptide that comprises a sequence that is at least 80 percent identical, preferably at least 85 percent and often 90 to 95 percent identical, more usually at least 99 percent identical, to a reference sequence over a comparison window of at least 20 nucleotide positions, frequently over a window of at least 25 to 50 nucleotides, wherein the percentage of sequence identity is calculated by comparing the reference sequence to the polynucleotide sequence, which may include deletions or additions that total 20 percent or less of the reference sequence, over the window of comparison. The reference sequence may be a subset of a larger sequence.

"Stringent conditions" refer to temperature and ionic conditions used in nucleic acid hybridization. The stringency required is nucleotide sequence dependent and also depends upon the various components present during hybridization. Generally, stringent conditions are selected to be about 5 to 20

degrees C lower than the thermal melting point (T_m) for the specific sequence at a defined ionic strength and pH. The T_m is the temperature (under defined ionic strength and pH) at which 50% of a target sequence hybridizes to a complementary probe.

"Substantially pure" means an object species is the predominant species present (i.e., on a molar basis, more abundant than any other individual macromolecular species in the composition), and a substantially purified fraction is a composition wherein the object species comprises at least about 50 percent (on a molar basis) of all macromolecular species present. Generally, a substantially pure composition means that about 80 to 90 percent or more of the macromolecular species present in the composition is the purified species of interest. The object species is purified to essential homogeneity (contaminant species cannot be detected in the composition by conventional detection methods) if the composition consists essentially of a single macromolecular species. Solvent species, small molecules (<500 Daltons), stabilizers (e.g., BSA), and elemental ion species are not considered macromolecular species for purposes of this definition.

"Suitable reaction conditions" are those conditions suitable for conducting a specified reaction using commercially available reagents. Such conditions are known or readily established by those of skill in the art for a variety of reactions. For example, suitable polymerase chain reaction (PCR) conditions include those conditions specified in U.S. Patents 4,683,202; 4,683,195; 4,800,159; and 4,965,188, each of which is incorporated herein by reference. As one example and not to limit the invention, suitable reaction conditions can comprise: 0.2 mM each dNTP, 2.2 mM $MgCl_2$, 50 mM KCl, 10 mM Tris-HCl, pH 9.0, and 0.1% Triton X-100.

"Telomere length regulatory protein" and "telomerase regulatory protein" refers to polypeptides involved in telomere metabolism and telomerase activity. Such proteins include telomerase, the protein components of telomerase, proteins that selectively bind nucleic acids containing telomere repeat sequences or telomeric ends, proteins required for telomere repair, maintenance, and/or elongation, and proteins necessary for expression or formation of active telomerase enzyme. Although the present invention relates to such proteins generally, mammalian telomerase, and particularly human telomerase, and related proteins are provided as preferred embodiments.

"Telomerase activity" refers to the ability of telomerase protein components to associate with one another and the RNA component of telomerase either *in vivo* or *in vitro* into a multi-component enzyme that can elongate telomeric DNA. A preferred assay method for detecting telomerase activity is the TRAP assay. See PCT patent publication No. 95/13381, *supra*. This assay measures

the amount of radioactive nucleotides incorporated into elongation products, polynucleotides, formed by nucleotide addition to a telomerase substrate or primer. The radioactivity incorporated can be measured as a function of the intensity of a band on a PhosphorImager™ screen exposed to a gel on which the radioactive products are separated. A test experiment and a control experiment can be compared by visually using the PhosphorImager™ screens. See also the commercially available TRAP-eze™ telomerase assay kit (Oncor); and Morin, 1989, *Cell* 59:521-529.

II. CLONING AND CHARACTERIZATION OF THE TPC2 AND TPC3 GENES

The present invention provides methods and reagents for regulating telomere length and modulating telomerase activity in mammalian cells as well as for detecting, diagnosing, and treating related diseases and conditions in humans and other mammals. The present invention arose in part out of an effort to clone the protein components of telomerase and other protein components of macromolecules that regulate telomere length and telomerase activity in human and other mammalian cells. These rare proteins and the mRNAs that encode these proteins are present in very low abundance in mammalian cells, necessitating the use of a novel mRNA isolation and identification method called "subtraction hybridization differential display."

In brief, this method involves obtaining mRNA from a first population of mammalian cells which contain the rare or low abundant protein of interest and from a second population of mammalian cells that contain 10- to 100-fold lower levels of the rare protein. The two mRNA populations are then individually used to generate cDNA preparations by reverse-transcription and second-strand synthesis to form first and second cDNA preparations. A detectable label is incorporated as well into the second cDNA preparation. The two cDNA preparations are then denatured and combined under conditions such that complementary strands of cDNA from the two cDNA preparations anneal to form a mixture of double-stranded and single-stranded cDNA. The mixture of cDNAs is then separated into two different populations, one comprising the label and one that does not, thereby forming an isolated, unlabeled preparation of cDNA that has been enriched for cDNA encoding the rare protein of interest. The steps of hybridization and separation can be repeated as often as desired, and the cDNA isolated after the separation step can be amplified by PCR, to provide cDNA preparations greatly enriched for the desired cDNA. Typically after two cycles of subtraction, cDNAs corresponding to abundant transcripts are depleted more

than 100-fold and low abundant transcripts are enriched in the subtracted cDNA libraries. The reproducibility of the method is excellent, and the method can be used to identify low abundant gene products such as those encoding telomere length and telomerase regulatory proteins.

To isolate cDNAs corresponding to telomere length and telomerase regulatory proteins, cDNA libraries were prepared from six different cells lines or tissues, three of which were "telomerase positive" (i.e., the cells express telomerase activity; the IDH4 and 293 cell lines, and testes tissue), and three of which were "telomerase negative" (i.e., the cells do not express telomerase activity; the HUVEC, BJ, and IMR-90 cell lines). These cDNA libraries were subjected to subtraction hybridization against the telomerase negative HUVEC cDNA library. Then, differential display was performed by first replicating each of the six subtracted cDNA libraries with either a single 5'-arbitrary primer or in a PCR with a 5'-arbitrary primer and a 3'-polydT primer, separating the replication products by gel electrophoresis, and identifying and isolating the differentially expressed products (identified visually as bands on a gel).

This process generated a number of differentially expressed cDNAs. Two of these cDNAs that were present in the cDNA libraries generated from the telomerase positive cell lines but not present (or present at much lower levels) in the telomerase negative cell lines, and that were later identified as originating from the 3'-ends of mRNA produced by the TPC2 and TPC3 genes, were isolated, cloned, and characterized by DNA sequence analysis. The DNA sequence analysis was used to design oligonucleotide primers that, in turn, were used to perform reverse-transcription and PCR (RT-PCR) on mRNA prepared from each of the same panel of six cell lines used to prepare the subtracted cDNA libraries. This RT-PCR experiment was designed to confirm that the mRNA corresponding to the putatively differentially expressed cDNAs is expressed at much higher levels in telomerase positive cell lines. The results were as predicted: the RT-PCR generated products of the predicted size; for the primers specific for the TPC2 mRNA, a substantial amount of product was generated using IDH4 mRNA, while lower amounts of product were generated using 293 and testes mRNA, and product was almost undetectable in mRNA prepared from HUVEC, BJ, and IMR-90 cells; for the primers specific for the TPC3 mRNA, product was generated only using mRNA from the telomerase positive cell lines.

To extend the analysis of the expression pattern of TPC2 and TPC3 in various cell lines and tissues, RT-PCR with primers specific for nucleotide sequences in the cDNAs corresponding to the differentially expressed TPC2 and TPC3 mRNAs was performed on a variety of cell lines. As a control, RT-PCR with

primers specific for nucleotide sequences in GAPDH mRNA (GAPDH is a "house-keeping" enzyme present in both telomerase positive and telomerase negative cell lines) was performed as well. In brief, the primers used for TPC2 were:

tpc-p1 5'-ATGGGGATTCCAGGGTGGAGCT-3', and

tpc-p4 5'-ACCTGCTCTCAGGGCCCACAAGT-3';

and the primers used for TPC3 were:

tpc-p13 5'-TAAGACAAAGAACAGGTCACAACA-3', and

tpc-p14 5'-ATTTGTGCTTAGAGGTCGTGCCAG-3'.

The RT-PCR was performed by making first strand cDNA made from total RNA with random hexamer primers and then PCR-amplifying the single-stranded cDNA with one of the two primer sets above, following the protocol of 16 to 28 cycles of PCR amplification (typically, 16 cycles for GAPDH mRNA, 25 cycles for TPC2 mRNA, and 27 cycles for TPC3 mRNA), with each cycle consisting of a step at 94 degrees C for 45 sec., 65 degrees C for 45 sec., and 72 degrees C for 90 sec. Other illustrative RT-PCR primers and conditions are shown in Parts C and D of the Examples below.

Figure 1, in parts A, B, and C, shows the results of RT-PCR analysis using primers specific for the TPC2 (Figure 1A) or TPC3 (Figure 1B) cDNA. Under these test conditions, TPC2 and TPC3 mRNA is absent or detectable only at very low levels in the telomerase negative cell lines tested (labeled "Mortal" in the Figure) and detectable in all (most at clearly detectable levels) telomerase positive cell lines tested (labeled "Immortal" in the Figure). These results, which show that TPC2 and TPC3 mRNA is present in testes tissue as well as most tumor cell lines but absent or present at lower abundance in normal cell lines, demonstrate how the methods of the invention for detecting and quantitating TPC2 and/or TPC3 gene products can be used to detect immortal cells, especially telomerase positive cancer cells, and so to diagnose cancer and other diseases and conditions in humans and other mammals. Figure 1C shows TPC3 mRNA levels normalized to GAPDH levels and illustrates the clear difference in TPC3 mRNA levels between mortal and immortal cells. This RT-PCR analysis also indicated that, as expected, the TPC2 and TPC3 mRNA is present in very low abundance even in telomerase positive cells (TPC2 or TPC3 mRNA amplification products detected after ~25 cycles; GAPDH or HPRT detected after ~15 or ~20 cycles, respectively). Confirmatory evidence for the low abundance of TPC2 mRNA in telomerase positive cells was obtained in the cloning of a cDNA corresponding to one-half of the full length TPC2 mRNA, where a primary screen of a lambda GT11 cDNA library from telomerase positive 293 cells showed that only one of ~1.4 million plaques was positive, indicating a very rare transcript.

Figure 2, in parts A, B, and C, is a bar graph showing the results of an RT-PCR analysis of hTR RNA and TPC2 and TPC3 mRNA levels as well as telomerase activity in a variety of cell lines. Figure 2A shows TPC2 and TPC3 mRNA levels normalized to GAPDH mRNA levels in various cell lines, all of which are telomerase positive except IMR-90, and demonstrates a correlation in the levels of these two telomere length and telomerase activity regulatory proteins. Figure 2B shows how TPC3 mRNA levels correlate with telomerase activity levels in a variety of cell lines. The IMR90, HTB-153, WI-38 VA13, KMSF, and T0 (unactivated T cells; note that T7 represents activated T cells) express no or only very low levels of telomerase activity. Figure 2C shows how hTR RNA levels correlate with telomerase activity levels in a variety of cell lines. The RT-PCR protocol for hTR RNA is described in Part D of the Examples; the nucleotide sequence of the hTR gene and transcribed RNA is shown in Figure 9.

Taken together, these Figures show that TPC2 and TPC3 mRNA levels as well as hTR levels correlate with telomerase activity levels in a variety of mortal and immortal cells lines. These results demonstrate how the methods of the invention for detecting TPC2 or TPC3 gene products can be used to detect immortal cells, especially telomerase positive cancer cells, and so to diagnose cancer and other diseases and conditions in humans and other mammals. These results also demonstrate the utility of the methods of the invention in which the detection or quantitation of TPC2 or TPC3 gene products, together with measurements of other factors, such as telomere length, telomerase activity, or hTR levels, can be used to identify immortal cells, such as cancer cells, or to evaluate the proliferative capacity of a cell.

The absence or very low abundance of the TPC2 and TPC3 gene products in telomerase negative mortal cells and the low but clearly detectable abundance of those gene products in telomerase positive immortal cells demonstrate the utility of the methods and reagents of the invention for detecting the presence gene products that encode proteins such as the protein components of telomerase and other proteins that regulate telomere length and telomerase activity in mammalian cells. A comparison of telomere length by mean terminal restriction fragment (mean TRF) analysis of immortal cell lines with TPC2 mRNA levels indicates that TPC2 mRNA levels are inversely related to telomere length. In one test, ten immortal cell lines with relatively high TPC2 mRNA levels had mean TRFs of ~2.5 to 5.0 kb, whereas two immortal cell lines with very low TPC2 mRNA levels had mean-TRFs of ~17.5 to 35 kb (probability of this difference arising by chance is less than 1%). In general, TPC2 mRNA levels also correlate well with telomerase activity levels in most cell lines tested.

Tests such as those described above can also be used to determine the mechanism of action by which the TPC2 and TPC3 gene products serve to regulate telomere length and telomerase activity. The tests on TPC2 provide some indication that the TPC2 gene product functions, at least in part, by acting as an indicator of telomere length, much like the yeast EST1 protein. TPC2 is up-regulated in most tumor cell lines and in testes cells and down-regulated in normal cell lines. However, some cell lines with apparently high levels of telomerase activity and very long telomeres have low levels of TPC2 mRNA. As noted above, however, telomerase positive cell lines that have relatively low TPC2 levels also have relatively high mean TRFs, i.e., skin melanoma LOX (~35.2 kb TRF), testes embryonic carcinoma Tera-1 (~27.0 kb), and lung carcinoma NCI-H23 (~17.5 kb). In contrast, skin melanoma lines SK MEL2 (~2.3 kb), SK MEL28 (~15.7 kb), SK MEL5 (~4.0 kb), and testes tissue (~15 kb) have relatively lower mean TRFs and relatively higher TPC2 mRNA levels. Because all of these cell lines have relatively high telomerase activity and high hTR levels, the tests indicate that cell lines with relatively long telomeres in general have low TPC2 mRNA levels, suggesting that the TPC2 protein may encode a protein with a telomere-sensing function. The analysis of TPC3 mRNA levels and telomerase activity in the same cell lines indicates that the TPC3 gene product may act as a core component of the telomerase enzyme.

Significant additional information regarding the mechanism of action of the TPC2 and TPC3 gene products in the regulation of telomere length and telomerase activity can be derived by analysis of the nucleotide sequence and corresponding amino acid sequence of the open reading frames of the corresponding genes. The subtraction hybridization differential display identification and cloning generated only cDNAs corresponding to the 3'-ends of the TPC2 and TPC3 mRNA gene products, but the nucleotide sequence information generated from those cDNAs provided a means to attempt to identify and isolate clones in cDNA libraries prepared from telomerase positive cell lines that comprise additional portions of the mRNA.

Full length cDNA for the TPC2 and TPC3 gene products was obtained by a variety of methods, including the screening of subtracted and other specialized libraries and the use of 5'-RACE. Initially, a lambda GT11 cDNA library containing human cDNA from 293 cells (a telomerase positive human-transformed kidney cell line available from ATCC) was screened to identify lambda clones that hybridized to the short TPC2 and TPC3 cDNAs obtained by subtraction hybridization differential display. Then, after screening additional cDNA libraries and combining fragments from various subclones, full length

open reading frames and genes were assembled into the plasmids pGRN92 (comprises the open reading frame of the TPC3 gene) and pGRN109 (comprises the open reading frame of the TPC2 gene).

For example, for TPC2, cDNA inserts in lambda clones were identified by screening with TPC2-specific probes and subcloned into plasmid pGEX and derivative vectors (Pharmacia) to yield plasmids that contained TPC2 cDNA in various reading frames to test expression products and obtain partial nucleotide sequence and deduced amino acid sequence information about the open reading frame of the TPC2 mRNA. In the case of TPC3, for example, cDNA fragments were cloned into pBluescript IIsk vector (Stratagene) to generate vectors for sequencing and analysis.

Figure 3 shows a restriction site and function map of the ~7.2 kb plasmid pGRN109, which contains an ~3.5 kb *Not*I-*Bst*EII restriction fragment that contains an ~3.3 kb open reading frame encoding the TPC2 protein (labeled "ORF" and "TPC2"). Figure 4 lists portions of the nucleotide sequence and deduced amino acid sequence of the TPC2 open reading frame corresponding to the human TPC2 gene, mRNA, and protein products. Figure 5 shows a restriction site and function map of the ~8 kb plasmid pGRN92, which contains an ~1.4 kb *Eco*RI-*Bam*HI restriction fragment that contains an ~1.1 kb open reading frame encoding the TPC3 protein (labeled "ORF" and "TPC3"). Figure 6 lists the nucleotide sequence and deduced amino acid sequence of the TPC3 open reading frame corresponding to the human TPC3 gene, mRNA, and protein products. The initiating methionine codon is marked with "***" and the stop codon with "—". Plasmid pGRN92 does not comprise nucleotides 1 - 82 shown in Figure 6.

Neither the TPC2 nor the TPC3 open reading frame or other gene sequences show significant homology to sequences in public databases other than to ESTs; however, both have motif signatures. TPC2 contains two WW domains and one L22 signature domain; TPC3 contains a homeobox domain. The "homeobox" is a protein domain of 60 amino acids (see Gehring, 1992, *Trends Biochem. Sci.* 17:277-280) first identified in a number of *Drosophila* homeotic and segmentation proteins and since found to be extremely well conserved in many animals, including vertebrates. This domain binds DNA through a helix-turn-helix type of structure. Proteins that contain homeobox domains are likely to play a role in development; most are known to be sequence specific DNA-binding transcription factors. Recent publications suggest that homeobox domains can bind RNA as well. See Dubnau and Struhl, 22 Feb. 1996, *Nature* 379:694. The homeobox domain in TPC3 is: LAMCTNLPEARVQVWFKNRRAKFR.

TPC2 contains two WW domains and an L22 ribosomal RNA signature domain. The ribosomal protein L22 is a protein component of the large ribosomal subunit that, in *E. coli*, binds 23S rRNA; the protein belongs to a family of ribosomal proteins. See Gantt *et al.*, 1991, *EMBO J.* 10:3073-3078. For TPC2, this domain is: SSSKVHSFGKRDQAIRRNPNVPVVV. The WW domain, also known as rsp5 or WWP, is a short conserved region in a number of unrelated proteins, among them dystrophin, responsible for Duchenne muscular dystrophy. The domain spans about 35 residues, can be repeated up to 4 times in some proteins, and has been shown to bind proteins with particular proline-motifs, [AP]-P-P-[AP]-Y, and so somewhat resembles SH3 domains. The WW domain is frequently associated with other proteins in signal transduction processes and appears to contain beta-strands grouped around four conserved aromatic positions, generally Trp; the name WWP derives from the presence of these conserved Trp and Pro residues. For TPC2, this domain is represented by three amino acid residue sequences: WSYGVCRDGRVFFINDQLRCTTWLHP; WFVLADYCLFYYKAEKKRSSXSIP; and WEEGFTEEGASYFIDHNQQTTAFRHP.

The availability of plasmids encoding the TPC2 and TPC3 open reading frames provides a wide variety of benefits, including the benefit of recombinant host cells that express recombinant gene products comprising TPC2 and/or TPC3 open reading frame sequences or sequences encoding products that react specifically with TPC2 and/or TPC3 gene products.

III. RECOMBINANT HOST CELLS

In one embodiment, the invention provides recombinant mammalian host cells containing:

(i) a recombinant or synthetic nucleic acid comprising at least about 10 to 15 to 25 to 100 or more contiguous nucleotides corresponding to an open reading frame sequence of a human gene TPC2 contained in a human DNA insert of an ~3.5 kb *Not*I-*Bst*EII restriction fragment of plasmid pGRN109; or

a synthetic or recombinant peptide or protein comprising at least about 6 to 10 to 15 to 25 to 100 or more contiguous amino acids corresponding to an amino acid sequence encoded by said open reading frame sequence; and

(ii) a recombinant or synthetic nucleic acid comprising at least about 10 to 15 to 25 to 100 or more contiguous nucleotides corresponding to an open reading frame sequence of a human gene TPC3 contained in a human DNA insert of an ~1.4 kb *Eco*RI-*Bam*HI restriction fragment of plasmid pGRN92; or

a synthetic or recombinant peptide or protein comprising at least about 6 to 10 to 15 to 25 to 100 or more contiguous amino acids corresponding to an amino acid sequence encoded by said open reading frame sequence of gene TPC3;

said TPC2 and TPC3 genes characterized in coding for proteins that regulate telomere length or modulate telomerase activity and are present in human or other mammalian cells that express telomerase activity.

Other mammalian host cells provided by the invention include those that comprise either or both TPC2- and TPC3-derived recombinant or synthetic nucleic acids, peptides, or proteins. Furthermore, the invention also provides such cells further modified to contain a synthetic or recombinant nucleic acid comprising at least about 10 to 15 to 25 to 100 or more contiguous nucleotides corresponding to a contiguous nucleotide sequence of human hTR located in an ~2.5 kb *Hin*dIII-*Sac*I restriction fragment of pGRN33 (ATCC 75926).

The recombinant host cells of the invention have application in many useful methods also provided by the invention. For example, the invention provides recombinant host cells comprising novel expression vectors with expression control sequences operatively linked to nucleotide sequences encoding amino acids in a sequence substantially identical to the proteins encoded by the human TPC2 or TPC3 genes, optionally with a recombinant hTR gene as well. These recombinant host cells are useful for producing recombinant human telomerase, for use in screens to identify agents that modulate telomerase activity or regulate telomere length, as well as for a variety of other purposes described below. The recombinant host cells of the invention can also be incorporated into the germ line and/or somatic tissues of non-human transgenic mammals, as well as be administered to mammals for therapeutic purposes.

Thus, genomic clones of a gene that regulates telomere length or telomerase activity, such as the human TPC2 or TPC3 gene, or recombinant versions thereof, including versions that encode mutein TPC2 or TPC3 gene products, may be used to construct homologous targeting constructs for generating cells and transgenic nonhuman animals having at least one functionally disrupted (or otherwise altered) allele. Guidance for construction of homologous targeting constructs may be found in the art, including: Rahemtulla *et al.*, 1991, *Nature* 353: 180; Jasin *et al.*, 1990, *Genes Devel.* 4:157; Koh *et al.*, 1992, *Science* 256:1210; Molina *et al.*, 1992, *Nature* 357:161; Grusby *et al.*, 1991, *Science* 253:1417; and Bradley *et al.*, 1992, *Bio/Technology* 10:534. See also U.S. Patent Nos. 5,464,764 and 5,487,992. Transgenic cells and/or transgenic non-human animals may be used to screen for antineoplastic agents and/or to screen for potential carcinogens, as inappropriate expression of a protein that regulates telomere

length or telomerase activity may result in a pre-neoplastic or neoplastic state or other disease state or condition. Homologous targeting can be used to generate so-called "knockout" mice, which are heterozygous or homozygous for an inactivated allele. Such mice may be sold commercially as research animals for investigation of immune system development, neoplasia, spermatogenesis, or as pets, or for animal products (foodstuff), or other purposes.

Chimeric transgenic mice are derived according to Hogan *et al.*, 1988, *Manipulating the Mouse Embryo: A Laboratory Manual*, Cold Spring Harbor Laboratory, and *Teratocarcinomas and Embryonic Stem Cells: A Practical Approach*, E.J. Robertson, ed., IRL Press, Washington, D.C. (1987). Embryonic stem cells are manipulated according to published procedures (*Teratocarcinomas and Embryonic Stem Cells: A Practical Approach*, E.J. Robertson, ed., IRL Press, Washington, D.C. (1987); PCT patent publication No. 96/22362; Zjilstra *et al.*, 1989, *Nature* 342:435; and Schwartzberg *et al.*, 1989, *Science* 246:799, each of which is incorporated herein by reference).

Additionally, a TPC2 or TPC3 cDNA or genomic clone may be used to construct transgenes for expressing polypeptides at high levels and/or under the transcriptional control of transcription control sequences which do not naturally occur adjacent to the gene (or vice-versa, i.e., the promoter of the TPC2 or TPC3 gene is positioned in front of a reporter gene for use in screening or other use). For example but not limitation, a constitutive promoter (e.g., an HSV-*tk* or *pgk* (phosphoglycerate kinase) promoter) or a cell-lineage specific transcriptional regulatory sequence (e.g., an CD4 or CD8 gene promoter/enhancer) may be operably linked to a protein encoding polynucleotide sequence to form a transgene (typically in combination with a selectable marker such as a *neo* gene expression cassette). Such transgenes can be introduced into cells (e.g., ES cells, hematopoietic stem cells, cancer cells), and transgenic cells, cell lines, and transgenic nonhuman animals may be obtained according to conventional methods therewith.

The recombinant host cells of the invention are often prepared using, or serve as a source of, valuable oligonucleotide and nucleic acid reagents provided by the present invention, such as the expression control vectors noted above. These nucleic acid reagents are described in more detail in the following section.

IV. OLIGONUCLEOTIDES AND NUCLEIC ACIDS

In another embodiment, the invention provides synthetic and recombinant oligonucleotides and nucleic acids in a variety of forms, i.e., isolatable, isolated, purified, or substantially pure, and for a variety of purposes, i.e., as probes or

primers, as polynucleotide plasmids and vectors for introducing recombinant gene products that regulate telomere length or modulate telomerase activity in mammalian host cells, as restriction fragments for creating useful nucleic acids, and as reagents for therapeutic, diagnostic, and other applications. Isolated or purified polynucleotides of the invention typically are less than ~10 kb in size. In particular, the invention provides recombinant or synthetic nucleic acids comprising at least about 10 to 15 to 25 to 100 or more contiguous nucleotides substantially identical or complementary in sequence to a contiguous nucleotide sequence located in either:

(i) an open reading frame sequence of a human gene TPC2 contained in a human DNA insert of an ~3.5 kb *Not*I-*Bst*EII restriction fragment of plasmid pGRN109; or

(ii) an open reading frame sequence of a human gene TPC3 contained in a human DNA insert of an ~1.4 kb *Eco*RI-*Bam*HI restriction fragment of plasmid pGRN92.

The novel oligonucleotide probes and primers of the invention typically comprise nucleotides in a sequence substantially identical or complementary to a sequence of nucleotides in a TPC2 or TPC3 gene or gene product to allow specific hybridization thereto in a complex mixture of nucleic acids. Nucleotide substitutions, deletions, and additions may be incorporated into the polynucleotides of the invention. Nucleotide sequence variation may result from sequence polymorphisms of various alleles, minor sequencing errors, and the like. The minimum length of a polynucleotide required for specific hybridization to a target sequence depends on several factors: G/C content, positioning of mismatched bases (if any), degree of uniqueness of the sequence as compared to the population of target polynucleotides, and chemical nature of the polynucleotide (e.g., methylphosphonate backbone, polyamide nucleic acid, phosphorothioate, etc.), among others.

The probes and primers of the invention have useful application in a variety of diagnostic, therapeutic, and other applications. Because they are expressed differentially between immortal human cells lines, TPC2 and TPC3 genes and gene products serve as telomerase activity and tumor cell markers. Oligonucleotides corresponding to unique TPC2 or TPC3 gene sequences can be used as primers or probes, may be attached to other nucleic acids, proteins, labels, etc., and are useful for a variety of purposes, including, for example, as diagnostic probes for tumor cells in clinical specimens. The oligonucleotides of the invention can be used as hybridization probes or PCR primers to detect the presence of TPC2 or TPC3 gene products, to diagnose a neoplastic disease characterized by

the presence of an elevated or reduced TPC2 or TPC3 mRNA level in cells, to perform tissue typing (i.e., identify tissues characterized by the expression of telomerase or TPC2 or TPC3 mRNA), and the like. Probes can be used to detect TPC2 or TPC3-specific nucleotide sequences in a DNA sample, such as for forensic DNA analysis or for diagnosis of diseases characterized by amplification, alteration, and/or rearrangements of the TPC2 or TPC3 genes. Certain preferred oligonucleotides of the invention typically comprise at least 8 to 10 to 15 to 25 to 99 to 250 to 1000 or more contiguous nucleotides capable of hybridizing under stringent hybridization conditions to nucleic acids corresponding to a nucleotide sequence in the ~3.5 kb *Not*I-*Bst*EII insert of pGRN109 or the ~1.4 kb *Eco*RI-*Bam*HI insert of pGRN92 and are useful as probes, primers, antisense therapeutics, and ribozyme therapeutics, for example.

Where expression of a polypeptide is not desired, polynucleotides of this invention need not encode a functional protein. Polynucleotides of this invention may serve as hybridization probes and/or PCR primers and/or LCR oligomers for detecting RNA or DNA sequences. Alternatively, polynucleotides of this invention may serve as hybridization probes or primers for detecting RNA or DNA sequences of related genes, for example, genes that encode structurally or evolutionarily related proteins. For such hybridization and other applications, such as those involving PCR, the polynucleotides of the invention need not encode a functional polypeptide. Thus, certain polynucleotides of the invention may contain substantial deletions, additions, nucleotide substitutions, and/or transpositions, so long as the ability of specific hybridization to or specific amplification of a TPC2 or TPC3 gene or mRNA gene product is retained.

As one example, antisense polynucleotides can include nucleotide substitutions, additions, deletions, or transpositions, so long as specific hybridization to the relevant target sequence, typically an mRNA, is retained as a functional property of the polynucleotide. Complementary antisense polynucleotides include soluble antisense RNA or DNA oligonucleotides that can hybridize specifically to mRNA species and genes and so prevent either transcription of the gene to produce the mRNA and/or translation of the mRNA. Antisense polynucleotides of various lengths may be used, although such antisense polynucleotides typically comprise a sequence of at least about 25 consecutive nucleotides that are substantially identical to a naturally occurring TPC2 or TPC3 gene sequence. Antisense polynucleotides may be produced from a heterologous expression cassette in a transfectant cell or transgenic cell, such as a transgenic pluripotent hematopoietic stem cell used to reconstitute all or part of the hematopoietic stem cell population of an individual. Alternatively, the

antisense polynucleotides may comprise soluble oligonucleotides that are administered to the external milieu, either in the culture medium *in vitro* or in the circulatory system or interstitial fluid *in vivo*. Soluble antisense polynucleotides present in the external milieu have been shown to gain access to the cytoplasm and inhibit translation of specific mRNA species. In some embodiments the antisense polynucleotides comprise methylphosphonate or other synthetic moieties. For general methods relating to antisense polynucleotides, see *Antisense RNA and DNA* (1988), D.A. Melton, Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

The inhibitory nucleic acid also can be a so-called "sense" or other nucleic acid, i.e., a triplex-forming nucleic acid. As one example, expression of recombinant TPC3 mRNA in a cancer cell line resulted in the inhibition of telomerase activity by over 90%. In this example, the entire ~1.1 kb coding sequence of the TPC3 gene was isolated as an *Eco*RI fragment (~2.1 kb) from vector pTATPC3.9 and inserted into the *Eco*RI site of mammalian expression vector pBBS212 to give rise to two vectors: pGRN111, in which the sense strand of the TPC3 gene is operatively linked to the myelo proliferative sarcoma virus (MPSV) promoter, and pGRN112, in which the antisense strand is operatively linked to the MPSV promoter. Vector pTATPC3.9 was constructed by ligation of TPC3 5'-RACE product (~2.1 kb) into pCRII vector (Invitrogen). The sense and antisense vectors, as well as control vector pBBS212, were used to transform HeTe7 cells by electroporation. The medium was changed to selection medium containing hygromycin (300 μg/ml) and puromycin (0.2 μg/ml) for four weeks to obtain individual clones. The individual clones were then isolated, expanded, and assayed for the expression of sense or antisense TPC3 gene product and vector transcription by RT-PCR. The positive clones were then assayed for telomerase activity using the TRAP assay, and mean TRF values were measured at different time points.

Figure 7 shows the results of the analysis of telomerase activity levels in recombinant HeTe7 cells expressing the sense or antisense mRNA of gene TPC3 or a control vector. As noted above, presence of the recombinant sense mRNA reduced telomerase activity markedly in these cells. Figure 8 shows the results of the analysis of telomere length in recombinant HeTe7 cells expressing the sense or antisense mRNA of gene TPC3 or a control vector. The recombinant TPC3 sense mRNA decreased the mean TRF in the cells. Thus, the recombinant TPC3 gene product can regulate not only telomerase activity but also telomere length in these cells. This experiment shows how the recombinant nucleic acids of the invention can be expressed by transfecting the cell with an expression vector comprising

expression control sequences operatively linked thereto. Fragments or analogs of TPC2 or TPC3 can also be expressed and function to compete with other active components of enzymes that regulate telomere length or telomerase activity. Assembly of ribonucleoproteins or other macromolecules with non-functional components results in non-functional complexes and subsequent decrease in associated activity, i.e., telomerase activity, telomere maintenance, and telomere length.

The expression vectors of the invention typically comprise expression control sequences operatively linked to a nucleotide sequence encoding amino acids in a sequence identical to a sequence of amino acids in a TPC2 or TPC3 protein gene product. The operably linked nucleotide sequence typically encodes at least 5 to 9 amino acids, or encodes all of or at least an active portion of the TPC2 or TPC3 proteins, or encode from 15 to 20 to 25 to 100 or more contiguous amino acids in a sequence selected from the amino acid sequences of TPC2 or TPC3, or variant but related sequences thereto. For example, useful TPC2 and TPC3 variant proteins include fusion proteins, in which all or a portion of the TPC2 or TPC3 protein is fused to peptide or polypeptide that imparts some useful feature, such as a binding site for use in affinity purification, i.e., a polyhistidine tag of about six histidine residues or the maltose binding protein. Preferably, these amino acid sequences occur in the given order of the naturally occurring proteins (in the amino-terminal to carboxy-terminal orientation) but may comprise other intervening and/or terminal sequences; generally such polypeptides are less than 1000 amino acids in length, more usually less than about 500 amino acids in lengths, and frequently about 200 amino acids in length. The degeneracy of the genetic code gives a finite set of polynucleotide sequences encoding these amino acid sequences; this set of degenerate sequences may be readily generated by hand or by computer using commercially available software (Wisconsin Genetics Software Package Release 7.0). These and other expression vectors of the invention have many useful applications, including in therapeutic methods of the invention as gene therapy vectors for modulating telomerase activity, either to activate or inhibit that activity, or for regulating telomere length, either to increase or decrease the length, in a target cell or tissue.

Thus, the gene therapy expression vectors of the invention include those that encode variants or "muteins" of the TPC2 and/or TPC3 proteins, i.e., express proteins that differ from TPC2 and/or TPC3 by deletion, substitution, and/or addition of one or more amino acids. The gene therapy vectors of the invention may also, however, encode other useful nucleic acids, such as hTR, or antisense nucleic acids or ribozymes that target the TPC2, TPC3, and/or hTR gene

products, i.e., mRNA and telomerase RNA. The vectors of the invention can also code for the expression of a protein which, when presented as an immunogen, elicits the production of an antibody that specifically binds to TPC2 or TPC3 proteins or cells expressing those proteins. Such vectors can also code for a structurally-related protein, such as a TPC2 or TPC3 protein fragment or analog. These vectors are useful in the therapeutic methods of the invention for treating or preventing diseases or conditions in which modulation of telomerase activity or telomere length can be of benefit. For example, in telomerase positive cancer cells, inhibition of telomerase activity can prevent telomere maintenance in those cells, inducing upon continued proliferation telomere loss, cell crisis, and death. For such purposes, the gene therapy vectors of the invention that express a non-functional TPC2 or TPC3 mutein or variant protein or other nucleic acid (i.e., over expression of TPC3 mRNA) that can inhibit telomerase formation or telomere elongation by telomerase activity in the cell, such as by competing for RNA component or protein components, inhibition of endogenous gene expression, or other means, are preferred.

Expression vectors of the invention comprise expression and replication signals compatible with the host cell of interest, i.e., sequences that facilitate transcription and translation (expression sequences) of the coding sequences, such that the encoded polypeptide product is produced. Construction of such polynucleotides is well known in the art and is described further in Maniatis *et al., supra.* For example, but not for limitation, such polynucleotides can include a promoter, a transcription termination site (polyadenylation site in eukaryotic expression hosts), a ribosome binding site, and, optionally, an enhancer for use in eukaryotic expression hosts, and, optionally, sequences necessary for replication of a vector. A typical eukaryotic expression cassette will include a polynucleotide sequence encoding a polypeptide linked downstream (i.e., in translational reading frame orientation; polynucleotide linkage) of a promoter such as the HSV, *tk, pgk,* metallothionein, or any of a wide variety of other promoters suitable for use in mammalian cells, optionally linked to an enhancer and a downstream polyadenylation site (e.g., an SV40 large T Ag poly A addition site). Expression vectors useful for expressing the recombinant TPC2, TPC3, and other proteins of this invention include viral vectors such as retroviruses, adenoviruses and adeno-associated viruses, i.e., for therapeutic methods, plasmid vectors such as pcDNA1 (Invitrogen, San Diego, CA), in which the expression control sequence comprises the CMV promoter, cosmids, liposomes, and the like. Viral and plasmid vectors are often preferred for transfecting mammalian cells.

The nucleic acid reagents of the invention also include reagents useful in identifying, isolating, and cloning nucleic acids that encode proteins that interact with TPC2 and TPC3 gene products as well as mammalian (i.e., mouse) homologs of human TPC2 and TPC3 genes. Homologous DNA can be readily identified by screening a genomic or cDNA clone library prepared from the mammalian cells of interest, such as a mouse, rat, rabbit, or other cells, i.e., in yeast artificial chromosomes, cosmids, or bacteriophage lambda (e.g., Charon 35), with a polynucleotide probe comprising a sequence of about at least 24 (or in the range of 15 to 30 or more) contiguous nucleotides (or their complement) of the cDNA sequences of TPC2 or TPC3 disclosed herein. Typically, hybridization and washing conditions are performed at varying degrees of stringency according to conventional hybridization procedures. Positive clones are isolated and sequenced. For illustration and not for limitation, a full length polynucleotide corresponding to the open reading frame sequences of the TPC2 and TPC3 genes can be labeled and used as a hybridization probe to isolate genomic clones from a murine or other mammalian genomic clone or cDNA library (i.e., those available from Promega Corporation, Madison, Wisconsin).

The nucleic acids of the invention can also be employed to isolate and identify gene products that interact with or bind to TPC2 and/or TPC3 gene products. The yeast "two-hybrid" system (see Chien *et al.*, 1991, *Proc. Natl. Acad. Sci.* (USA) 88:9578) utilizes expression vectors that encode the predetermined polypeptide sequence as a fusion protein and is used to identify protein-protein interactions *in vivo* through reconstitution of a transcriptional activator (see Fields and Song, 1989, *Nature* 340:245). Usually the yeast Gal4 transcription protein, which consists of separable domains responsible for DNA-binding and transcriptional activation, serves as the transcriptional activator. Polynucleotides encoding two hybrid proteins, one consisting of the yeast Gal4 DNA-binding domain fused to a polypeptide sequence of a first protein and the other consisting of the Gal4 activation domain fused to a polypeptide sequence of a second protein (either the first or second protein typically is a number of different proteins to be screened for ability to interact specifically with the other protein), are constructed and introduced into a yeast host cell. Intermolecular binding, if any, between the two fusion proteins reconstitutes the Gal4 DNA-binding domain with the Gal4 activation domain, which leads to the transcriptional activation of a reporter gene (e.g., *lacZ*, *HIS3*) operably linked to the Gal4 binding site. Typically, the two-hybrid method is used to identify novel polypeptide sequences which interact with a known protein.

The invention also provides two- and three-hybrid systems, typically in the form of polynucleotides encoding a first hybrid protein comprising either TPC2 or TPC3, a second hybrid protein, and a reporter gene, wherein said polynucleotide(s) are either stably replicated or introduced for transient expression. The host organism can be a yeast cell (e.g., *Saccharomyces cervisiae*) in which the reporter gene transcriptional regulatory sequence comprises a Gal4-responsive promoter (binding site). Yeast cells comprising (1) an expression cassette encoding a Gal4 DNA binding domain (or Gal4 activator domain) fused to a binding fragment of TPC2 or TPC3 protein; (2) an expression cassette encoding a Gal4 DNA activator domain (or Gal4 binding domain, respectively) fused to a member of a cDNA library; and (3) a reporter gene (e.g., beta-galactosidase) comprising a cis-linked Gal4 transcriptional response element, can be used to screen cDNAs to identify those that encode polypeptides that bind to TPC2 and/or TPC3 proteins specifically. Yeast two-hybrid systems may be used to screen a mammalian (typically human) cDNA expression library, such as, for example, a cDNA library produced from human mature B cell line (Namalwa) mRNA (see Ambrus *et al.*, 1993, *Proc. Natl. Acad. Sci.* (U.S.A.)). Once cDNAs encoding such interacting polypeptides are identified, the resulting polypeptides can be cloned, characterized, and used to screen compounds to identify compounds that can inhibit the binding interaction.

Notwithstanding the many and diverse application of the oligonucleotide and nucleic acid reagents of the invention, one important application relates to the production of recombinant peptides and proteins of the invention, as discussed in the following section.

V. PEPTIDES AND PROTEINS

In another embodiment, the present invention provides peptides, proteins, antibodies, and enzymes relating to genes and gene products that regulate telomere length and telomerase activity in mammalian cells. In particular, the invention provides synthetic or recombinant peptides or proteins comprising at least about 6 to 10 to 15 to 25 to 100 or more contiguous amino acids identical in sequence to an amino acid sequence encoded by an open reading frame sequence of a human gene located in either:

(i) an ~3.5 kb *Not*I-*Bst*EII restriction fragment of plasmid pGRN109; or

(ii) an ~1.4 kb *Eco*RI-*Bam*HI restriction fragment of plasmid pGRN92.

The present invention provides the peptides and proteins encoded by the TPC2 and TPC3 genes, as well as fragments and analogs thereof, in isolatable form from eukaryotic or prokaryotic host cells expressing recombinant TPC2

and/or TPC3 protein, or from an *in vitro* translation system, as well as in purified and substantially pure form from synthesis *in vitro* or by purification from recombinant host cells or by purification of the naturally occurring proteins using antibodies or other reagents of the invention. Methods for expression of heterologous proteins in recombinant hosts, chemical synthesis of polypeptides, and *in vitro* translation are well known in the art and are described further in Maniatis *et al.* and Berger and Kimmel, *supra*. Such proteins have application in methods for reconstituting *in vitro* telomerase or other enzymatic activities that maintain telomeres and regulate telomere length. These methods in turn have application in screens for therapeutic agents, for diagnostic tests, and for other applications.

Because they are expressed differentially between immortal human cells lines, TPC2 and TPC3 genes and gene products serve as telomerase activity and tumor cell markers. Polypeptides having the full or partial amino acid sequence of TPC2 or TPC3 proteins are useful, for example, in the production of antibodies against TPC2 or TPC3 proteins and that are useful in the detection of TPC2 or TPC3 proteins in tumor cells. The invention provides purified TPC2 and TPC3 proteins having an amino acid sequence substantially identical to the amino acid sequences encoded by the open reading frames of the TPC2 and TPC3 genes. Such genes include human allelic variants or mammalian cognate genes that can be obtained in accordance with and using the reagents provided by the present invention.

The invention also provides TPC2 and TPC3 protein analogs, non-naturally occurring polypeptides comprising at least 5 to 10 to 15 to 20 to 25 to 100 or more amino acids in a contiguous sequence selected from the amino acid sequences of the TPC2 and TPC3 proteins but include one or more deletions or additions of amino acids, either at the amino- or carboxy-termini, or internally, or both; analogs may further include sequence transpositions. Analogs may also comprise amino acid substitutions, preferably conservative substitutions. Analogs include active fragments as well as various muteins. For example, single or multiple amino acid substitutions (preferably conservative amino acid substitutions) may be made in the naturally occurring sequence. Preferred amino acid substitutions include those that: (1) reduce susceptibility to proteolysis, (2) reduce susceptibility to oxidation, (3) alter post-translational modification of the analog, possibly including phosphorylation, and (4) confer or modify other physicochemical or functional properties of such analogs. TPC2 or TPC3 protein analogs can be immunogenic for TPC2 or TPC3 proteins, i.e., when presented as an immunogen, the analog elicits the production of an antibody that specifically binds to TPC2 or

TPC3 proteins. Active fragments can be identified empirically by generating fragments of the full length protein by deletion from either the amino-terminus or the carboxy-terminus or both, and testing the resulting fragments for activity.

Conservative amino acid substitution is a substitution of an amino acid by a replacement amino acid which has similar characteristics (e.g., those with acidic properties: Asp and Glu). A conservative (or synonymous) amino acid substitution does not substantially change the structural characteristics of the parent protein (e.g., a replacement amino acid should not tend to break a helix that occurs in the parent sequence, or disrupt other types of secondary structure that characterizes the parent sequence). Examples of art-recognized polypeptide secondary and tertiary structures are described in *Proteins, Structures and Molecular Principles* (1984), Creighton (ed.), W.H. Freeman and Company, New York; *Introduction to Protein Structure* (1991), C. Branden and J. Tooze, Garland Publishing, New York, NY; and Thornton *et al.*, 1991, *Nature* 354:105; which are incorporated herein by reference. The following six groups each contain amino acids that are conservative substitutions for one another: (1) Alanine (A), Serine (S), Threonine (T); (2) Aspartic acid (D), Glutamic acid (E); (3) Asparagine (N), Glutamine (Q); (4) Arginine (R), Lysine (K); (5) Isoleucine (I), Leucine (L), Methionine (M), Valine (V); and (6) Phenylalanine (F), Tyrosine (Y), Tryptophan (W).

Analogs may include heterologous sequences generally linked at the amino- or carboxy-terminus, wherein the heterologous sequence(s) confer a functional property to the resultant analog not shared by the native protein. Such analogs are referred to as fusion proteins and for purposes of the present invention typically comprise a TPC2 or TPC3 protein or analog and an additional peptide or protein moiety. Fusion proteins usefully combine properties of two different polypeptides or proteins, and can be used, for example, to confer a label, such as a polyhistidine polypeptide or a maltose binding protein, useful in affinity isolation of the fusion protein or to protect the fusion protein from degradation inside a cell. The fusion protein may comprise a linker peptide with desired properties, for example, a peptidase site that renders the TPC2 or TPC3 protein or analog cleavable from the remainder of the fusion protein. The fusion protein can also confer an antigenic epitope to the TPC2 or TPC3 protein of interest; antibodies that bind the epitope could then be used to immunoprecipitate the fusion protein for purification or to identify associated proteins.

Thus, the invention provides recombinant fusion proteins in which all or a portion of the TPC2 or TPC3 protein is fused to another polypeptide or protein of interest. For example, plasmids pGRN103, pGRN104, pGRN106, and pGRN110

are expression plasmids of the invention that code for the expression of novel fusion proteins of the invention that comprise a portion of either TPC2 or TPC3 protein and maltose binding protein (MBP). These vectors were created using the commercially available pMALc2 expression vector and system (New England Biolabs). Plasmid pGRN103 encodes a fusion protein comprising the amino-terminal portion of TPC3 protein and MBP and was prepared by replacing the *Xmn*I-*Pst*I restriction fragment of plasmid pMALc2 with the *Pvu*II-*Pst*I restriction fragment of plasmid pGRN92. Plasmid pGRN104 encodes a fusion protein comprising the carboxy-terminal portion of TPC3 protein and MBP and was prepared by replacing the *Ecl*136II-*Bam*HI restriction fragment of plasmid pMALc2 with the *Bsp*EI (treated with Klenow in the presence of dCTP and dGTP only) - *Bam*HI restriction fragment of plasmid pGRN92. Plasmid pGRN106 encodes a fusion protein comprising the amino-terminal portion of TPC2 protein and MBP and was prepared by replacing the *Sal*I-*Pst*I restriction fragment of plasmid pMALc2 with a *Sal*I-*Sse*8387I restriction fragment that can be isolated from plasmid pGRN109. Plasmid pGRN110 encodes a fusion protein comprising the carboxy-terminal portion of TPC2 protein and MBP and was prepared by inserting a restriction fragment containing the carboxy-terminal portion of the open reading frame of TPC2 into plasmid pMALc2 such that the fusion protein shown below results from expression of the plasmid in *E. coli* W3110 cells (only the ends of the MBP and TPC2 proteins at the junction region are shown):

```
            Maltose Binding Protein
**************************************************><**Junction*
ProAsnIleProGlnMetSerAlaPheTrpTyrAlaValArgThrAlaValIleAsnAla
AlaSerGlyArgGlnThrValAspGluAlaLeuLysAspAlaGlnThrAsnSerSerSer
AsnAsnAsnAsnAsnAsnAsnAsnAsnLeuGlyIleGluGlyArgIleSerGluPhe
                                    TPC2 Protein
***Junction***><**************************************************
AlaAlaAlaSerThrLeuAspLeuLysMetThrGlyArgAspLeuLeuLysAspArgSer
**********************************************************
LeuLysProValLysIleAlaGluSerAspThrAspValLysLeuSerIlePheCysGlu
```

These and other fusion proteins of the invention can be isolated in accordance with standard procedures and then used to immunize animals, i.e., mouse and rabbits, for the production of polyclonal antisera and monoclonal antibodies, as described in the following section.

TPC2 or TPC3 proteins, analogs, peptides, and polypeptides can also be prepared by chemical synthesis using well known methods. For example, various peptides with amino acid sequences corresponding to sequences of the TPC2 and

TPC3 proteins can be chemically synthesized *in vitro* and used to generate antibodies that specifically bind to TPC2 and/or TPC3 proteins. Illustrative peptides of the invention include RGLKRQSDERKRDRE and KVTSPLQSPTKAKPK, which have been chemically synthesized *in vitro* and used to immunize animals to generate antibodies specific for TPC3 protein. Such peptides may correspond to structural and functional domains identified by comparison of the nucleotide and/or amino acid sequence data of a gene or protein to public or other sequence databases. Computerized comparison methods can be used to identify sequence motifs or predicted protein conformation domains that occur in other proteins of known structure and/or function. See *Proteins, Structures and Molecular Principles* (1984), Creighton (ed.), W.H. Freeman and Company, New York, incorporated herein by reference. Methods to identify protein sequences that fold into a known three-dimensional structure are known. See Bowie *et al.*, 1991, *Science* 253:164. Recognized sequence motifs and structural conformations may be used to define structural and functional domains. Computer programs GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package (Genetics Computer Group, 575 Science Dr., Madison, WI) can be used to identify sequences in databases, such as GenBank/EMBL, that have regions of homology. Neural network methods, whether implemented in hardware or software, may be used to: (1) identify related protein sequences and nucleotide sequences, and (2) define structural or functional domains in polypeptides. See Brunak *et al.*, 1991, *J. Mol. Biol.* 220:49, incorporated herein by reference.

Thus, one class of preferred peptides and proteins of the invention are fragments of the TPC2 or TPC3 proteins having amino- and/or carboxy-termini corresponding to amino acid positions near functional domain borders. Alternative fragments may also be prepared. The choice of the amino- and carboxy-termini of such fragments rests with the discretion of the practitioner and is based on considerations such as ease of construction, stability to proteolysis, thermal stability, immunological reactivity, amino- or carboxyl-terminal residue modification, or other considerations.

The immunogenic peptides and proteins of the invention can be used in therapeutic immunization and vaccination procedures. The invention therefore provides a method of immunizing a subject, as well as vaccines useful in the method, against cells that maintain telomeres and express telomerase activity, such as cancer cells, that comprise administering an immunostimulating amount of such peptides or proteins of the invention.

Peptides and proteins of the invention are suitably obtained in substantially pure form if at least about 50 percent (w/w) or more pure and

substantially free of interfering proteins and contaminants. Preferably, these polypeptides are isolated or synthesized in a purity of at least 80 percent (w/w) or, more preferably, in at least about 95 percent (w/w), and are substantially free of other proteins or contaminants.

One important application of the peptides and proteins of the invention is the generation of antibodies that specifically bind to TPC2 or TPC3 proteins, as discussed in the following section.

VI. ANTIBODIES

The proteins and peptides of the invention can also be used to generate antibodies specific for TPC2 or TPC3 proteins, or for particular epitopes on those proteins. TPC2 or TPC3 proteins, fragments thereof, or analogs thereof, can be used to immunize an animal for the production of specific antibodies. For example, but not for limitation, a recombinantly produced fragment of a TPC2 or TPC3 protein or a fusion protein can be injected into a mouse along with an adjuvant following immunization protocols known to those of skill in the art so as to generate an immune response. Alternatively, or in combination with a recombinantly produced polypeptide, a chemically synthesized peptide having an amino acid sequence corresponding to a TPC2 or TPC3 protein may be used as an immunogen to raise antibodies which bind a TPC2, TPC3, or another telomere- or telomerase-related protein. Immunoglobulins that bind the target protein with a binding affinity of at least about $1 \times 10^6\ M^{-1}$ can be harvested from the immunized animal as an antiserum, and may be further purified by immunoaffinity chromatography or other means.

Additionally, spleen cells can be harvested from the immunized animal (typically rat or mouse) and fused to myeloma cells to produce a bank of monoclonal antibody-secreting hybridoma cells. The bank of hybridomas can be screened for clones that secrete immunoglobulins that bind the protein of interest specifically, i.e., with an affinity of at least $1 \times 10^7\ M^{-1}$. Animals other than mice and rats may be used to raise antibodies; for example, goats, rabbits, sheep, and chickens may also be employed to raise antibodies reactive with a TPC2 or TPC3 protein. Transgenic mice having the capacity to produce substantially human antibodies also may be immunized and used for a source of antiserum and/or for making monoclonal antibody secreting hybridomas.

Thus, the invention provides polyclonal and monoclonal antibodies that specifically bind to TPC2 or TPC3 proteins. Bacteriophage antibody display libraries may also be screened for phage able to bind peptides and proteins of the invention specifically. Combinatorial libraries of antibodies have been generated

in bacteriophage lambda expression systems and may be screened as bacteriophage plaques or as colonies of lysogens. For general methods to prepare antibodies, see *Antibodies: A Laboratory Manual* (1988), E. Harlow and D. Lane, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, incorporated herein by reference.

These antibodies can in turn be used to isolate TPC2 or TPC3 proteins from normal or recombinant cells and so can be used to purify the proteins as well as other proteins associated therewith. Such antibodies are useful in the detection of TPC2 or TPC3 proteins in samples and in the detection of cells comprising TPC2 or TPC3 proteins in complex mixtures of cells. Such detection methods have application in screening, diagnosing, and monitoring diseases and other conditions, such as cancer, pregnancy, or fertility, because the TPC2 and TPC3 proteins are present in most cells capable of elongating telomeric DNA and expressing telomerase activity and are present in those cells at levels significantly higher than the levels observed in telomerase negative cells.

For some applications of the antibodies of the invention, such as identifying immuno-crossreactive proteins, the desired antiserum or monoclonal antibody(ies) is/are not monospecific. In these or other instances, it may be preferable to use a synthetic or recombinant fragment of a TPC2 or TPC3 protein as an antigen rather than the entire protein. More specifically, where the object is to identify immuno-crossreactive polypeptides that comprise a particular structural moiety, such as a DNA-binding domain, it is preferable to use as an antigen a fragment corresponding to part or all of a commensurate structural domain in the TPC2 or TPC3 protein.

Cationized or lipidized antibodies reactive with TPC2 or TPC3 can be used therapeutically to treat or prevent diseases of excessive or inappropriate expression (e.g., neoplasia) of these proteins and the processes regulated thereby. Other methods of the invention are discussed in the following section.

VII. METHODS

The various reagents of the invention described above have a wide variety of applications. The provision of polynucleotides capable of hybridizing to TPC2 or TPC3 cDNA and antibodies that specifically bind to TPC2 or TPC3 proteins allows one to detect expression of TPC2 and TPC3 in cells. The detection of TPC2 or TPC3 gene expression in cells suspected of being cancerous is useful in the diagnosis of cancer. Accordingly, this invention provides methods of detecting TPC2 or TPC3 mRNA or protein in a cell by hybridization or immunoassay methods. Hybridization methods can involve any of the routine methods

including Northern blotting; Southern hybridization; amplification of target or probe nucleic acids by PCR, b-DNA, antibodies labeled with enzymes, LCR, Q-beta replicase, or 3SR; and the like, may also be used.

The polynucleotide sequences of the present invention can be used for forensic identification of individual humans, such as for identification of decedents, determination of paternity, criminal identification, and the like. The invention also provides TPC2 or TPC3 polynucleotide probes for diagnosis of disease states (e.g., neoplasia or pre-neoplasia) by detection of a TPC2 or TPC3 mRNA or rearrangements or amplification of the TPC2 or TPC3 gene in cells explanted from a patient, or detection of a pathognomonic TPC2 or TPC3 allele. Cells which contain an altered amount of TPC2 or TPC3 mRNA as compared to non-neoplastic or non-diseased cells of the same cell type(s) can be identified as candidate diseased cells in accordance with the methods of the invention. Similarly, the detection of pathognomonic rearrangements or amplification of the TPC2 or TPC3 gene locus or closely linked loci in a cell sample will identify the presence of a pathological condition or a predisposition to developing a pathological condition (e.g., cancer, genetic disease).

The isolation of three telomerase-related and telomere length regulatory components, TPC2, TPC3, and hTR, allows the production of recombinant telomerase comprising one or more of these components. In one method, recombinant telomerase is produced by expressing a TPC2 or TPC3 protein or active TPC2 or TPC3 analog and/or recombinant hTR in a cell. In another, telomerase is re-constituted *in vitro*. The recombinant RNA component of telomerase can be, for example, an RNA molecule derived from the sequence encoded by the ~2.5 kb *Hin*dIII-*Sac*I insert of pGRN33 (ATCC 75926). Recombinant telomerase is useful, for example for screening assays to determine whether a compound modulates telomerase activity.

Telomerase- and telomere length-modulating agents which reduce a cell's capacity to repair telomere DNA damage (e.g., by inhibiting endogenous naturally occurring telomerase) are candidate antineoplastic agents. Candidate antineoplastic agents are then tested further for antineoplastic activity in assays which are routinely used to predict suitability for use as human antineoplastic drugs. Examples of these assays include, but are not limited to, assays to measure the ability of the candidate agent (1) to inhibit anchorage-independent transformed cell growth in soft agar, (2) to reduce tumorigenicity of transformed cells transplanted into *nu/nu* mice, (3) to reverse morphological transformation of transformed cells, (4) to reduce growth of transplanted tumors in *nu/nu* mice, (5) to inhibit formation of tumors or pre-neoplastic cells in animal models of

spontaneous or chemically-induced carcinogenesis, and (6) to induce a more differentiated phenotype in transformed cells.

Administration of an efficacious dose of an agent capable of specifically inhibiting telomere-maintenance or telomerase activity to a patient can be used as a therapeutic or prophylactic method for treating pathological conditions (e.g., cancer, inflammation, lymphoproliferative diseases, autoimmune disease, neurodegenerative diseases, and the like), which are effectively treated by modulating telomerase activity and telomere length. Additional embodiments directed to modulation of neoplasia or cell death include methods that employ specific inhibitory nucleic acids, e.g., sense or antisense polynucleotides corresponding to nucleotide sequences encoding TPC2, TPC3, or a cognate mammalian TPC2 or TPC3 protein.

The foregoing description of the preferred embodiments of the present invention has been presented for purposes of illustration and description and is not intended to be exhaustive or to limit the invention to the precise form disclosed but instead to illuminate the many modifications and variations possible in light of the invention and description and to include such modifications and variations as may be apparent to a person skilled in the art in light of this description within the scope of this invention and the claims thereto. All publications and patent documents cited in this application are incorporated by reference in their entirety for all purposes to the same extent as if each individual publication or patent document were so individually denoted.

VIII. EXAMPLES

The following examples are given to illustrate but not limit the invention. Generally, the nomenclature used herein and many of the laboratory procedures in cell culture, molecular genetics, and nucleic acid chemistry and hybridization described below are those well known and commonly employed in the art. All percentages given throughout the specification and examples are based upon weight unless otherwise indicated. All protein molecular weights are based on mean average molecular weights unless otherwise indicated.

A. Methods In Molecular Genetics

Standard techniques are used for recombinant nucleic acid methods, polynucleotide synthesis, *in vitro* polypeptide synthesis, microbial culture and transformation (e.g., electroporation), and the like. Generally enzymatic reactions and purification steps using commercially available starting materials are performed according to the manufacturer's specifications. The techniques and

procedures are generally performed according to conventional methods in the art and various general references (see, generally, Sambrook *et al. Molecular Cloning: A Laboratory Manual*, 2d ed. (1989); Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., incorporated herein by reference) referenced herein.

Oligonucleotides can be synthesized on an Applied Bio Systems or other commercially available oligonucleotide synthesizer according to specifications provided by the manufacturer. Polynucleotide primers may be prepared using any suitable method, such as, for example, the phosphotriester and phosphodiester methods, or automated embodiments thereof. In one such automated embodiment, diethylphosphoramidites are used as starting materials and may be synthesized as described by Beaucage *et al.*, 1981, *Tetrahedron Letters* 22:1859, and U.S. Patent No. 4,458,066.

Methods for PCR amplification are known in the art (*PCR Technology: Principles and Applications for DNA Amplification*, Ed. Erlich, Stockton Press, New York, NY (1989); *PCR Protocols: A Guide to Methods and Applications*, eds. Innis, Gelfland, Sninsky, and White, Academic Press, San Diego, CA (1990); Mattila *et al.*, 1991, *Nucleic Acids Res.* 19:4967; Eckert and Kunkel, 1991, *PCR Methods and Applications* 1:17; and the U.S. Patents noted above. Optimal PCR and hybridization conditions will vary depending upon the sequence composition and length(s) of the targeting polynucleotide(s) primers and target(s) employed, and the experimental method selected by the practitioner. Various guidelines may be used to select appropriate primer sequences and hybridization conditions (see, Sambrook *et al., supra*). Generally PCR is carried out in a buffered aqueous solution, preferably at a pH of 7-9, most preferably about 8. The deoxyribonucleoside triphosphates dATP, dCTP, dGTP, and TTP are also added to the synthesis mixture in adequate amounts, and the resulting solution is heated to about 85-100 degrees C for about 1 to 10 minutes, preferably from 1 to 4 minutes. After this heating period, the solution is allowed to cool to about 20-40 degrees C, for primer hybridization. To the cooled mixture is added an agent for polymerization, and the reaction is allowed to occur under conditions known in the art. This synthesis reaction may occur at from room temperature up to a temperature just over which the agent for polymerization no longer functions efficiently. Thus, for example, if a heat-labile DNA polymerase is used as the agent for polymerization, the synthesis temperature is generally no greater than about 45 degrees C. The agent for polymerization may be any compound or system that will function to accomplish the synthesis of primer extension products, including enzymes. Suitable enzymes for this purpose include, for

example, *E. coli* DNA polymerase I or the Klenow fragment thereof, *Taq* DNA polymerase, and other available DNA polymerases.

The newly synthesized strand and its complementary nucleic acid strand form double-stranded molecules used in the succeeding steps of the process. In the next step, the strands of the double-stranded molecule are separated using any of the procedures described above to provide single-stranded molecules. The steps of strand separation and extension product synthesis can be repeated as often as needed to produce the desired quantity of the specific nucleic acid sequence. The amount of the specific nucleic acid sequence produced will accumulate in an exponential fashion.

B. Subtractive Hybridization Differential Display

Both the subtractive hybridization method and the differential display method have disadvantages for isolating rare mRNAs that are differentially expressed. Subtractive hybridization can be useful for enriching a pool of non-abundant cDNA species, but conventional screening of the resultant library(ies), even if PCR amplified, is biased in favor of identifying species that are still abundant within the selected non-abundant cDNA pool, making difficult the isolation of very rare cDNA species with a conventional subtractive hybridization enrichment protocol. Differential display of mRNA amplified by PCR is biased by the initial abundance of the various mRNA species and often under-represents or fails to detect rare mRNA species among the many mRNA species that are more abundant and not substantially differentially expressed.

The present invention provides a subtractive hybridization differential display method that is particularly preferred for isolating rare mRNAs, such as those expressed by the TPC2 and TPC3 genes. In brief, this method comprises the steps of: (1) one or more cycles of subtractive hybridization of two cDNA populations to generate a population of subtracted cDNA that is selectively enriched for cDNA species of low abundance mRNAs that are present at higher levels in one of the two cDNA populations, and (2) differential display of the cDNA on an electrophoretic gel and recovery of individual differentially expressed cDNAs by recovery from the gel. PCR amplification, under suitable PCR conditions, of said subtracted cDNA population with a 5' primer of arbitrary nucleotide sequence and optionally with a 3' primer comprising poly(dT) and/or poly(dT) and two or more arbitrary nucleotides at the 3' end to generate PCR products is typically used to replicate or amplify a subtracted library.

To accomplish the initial step(s) of subtractive hybridization, RNA prepared by conventional methods from a first cell population and RNA from a

second cell population are separately reverse-transcribed and second-strand synthesized to form two pools of double-stranded cDNA, a *tester* pool comprising sequences of the mRNA species(s) for which enrichment is desired, and a *driver* pool comprising the sequences to be subtracted from the tester pool. The two pools may be fragmented by endonuclease digestion (restriction endonuclease or non-specific endonuclease) if desired to degrade cDNA consisting of tandem repeated sequences and to enhance hybridization efficiency. The driver pool is labeled, such as by photobiotinylation or attachment of another suitable recoverable label. The driver pool and tester pool are denatured and mixed together in a reaction mixture under hybridization conditions and incubated for a suitable hybridization period. The reaction mixture is contacted with a ligand which binds the recoverable label on the driver cDNA and which can be readily recovered from the reaction mixture (e.g., using avidin attached to magnetic beads), such that a substantial fraction of the driver cDNA and any tester cDNA hybridized thereto is selectively removed from the reaction mixture.

The remaining reaction mixture is enriched for tester cDNA species that are preferentially expressed in the first cell population as compared to the second cell population. The enriched (subtracted) tester cDNA pool may be subjected to one or more additional rounds of subtractive hybridization with a pool of labeled driver cDNA, which may be substantially identical to the initial pool of driver cDNA or which may represent a different cell population having mRNA species which are desired to be subtracted from the subtracted tester cDNA pool. A variety of means for accomplishing the subtractive hybridization(s) and suitable methodological guidance are available to the artisan. See Lee *et al.*, 1991, *Proc. Natl. Acad. Sci.* (U.S.A.) 88:2825; Milner *et al.*, 1995, *Nucleic Acids Res.* 23:176; Luqmani *et al.*, 1994, *Anal. Biochem.* 222: 102; Zebrowski *et al.*, 1994, *Anal. Biochem.* 222:285; Robertson *et al.*, 1994, *Genomics* 23:42; Rosenberg *et al.*, 1994, *Proc. Natl. Acad. Sci.* (U.S.A.) 91:6113; Li *et al.*, 1994, *Biotechniques* 16:722; Hakvoort *et al.*, 1994, Nucleic Acids Res. 22:878; Satoh *et al.*, 1994, *Mutat. Res.* 316:25; Marechal *et al.*, 1993, *Anal. Biochem.* 208:330; El-Deiry *et al.*, 1993, *Cell* 75:817; Hara *et al.*, 1991, *Nucleic Acids Res.* 19:7097; and Herfort and Garber, 1991, *Biotechniques* 11:598, each of which is incorporated herein by reference.

After the subtractive hybridization is completed, the subtracted tester cDNA is subjected to differential display. The general strategy involves amplification of cDNAs from the subtracted tester cDNA pool by PCR using one or a set of arbitrary sequence primers. Arbitrary primers are selected according to various criteria at the discretion of the practitioner so that each will amplify only a fraction of the DNAs in the subtracted cDNA pool so that the amplification

products can be resolved and individually recovered on a separation system, such as a polyacrylamide gel. In part because the number and complexity of cDNA species represented in any particular subtracted tester pool may vary considerably depending upon the nature and complexity of the driver and tester pools, the selection of arbitrary primers and their sequence(s) is determined by the practitioner with reference to the literature. See Linskens *et al.*, 1995, *Nucleic Acids Res.* 23 (16): 3244-3251; Liang *et al.*, 1993, *Nucleic Acids Res.* 21:3269; Utans *et al.*, 1994, *Proc. Natl. Acad. Sci.* (U.S.A.) 91:6463; Zimmermann *et al.*, 1994, *Proc. Natl. Acad. Sci.* (U.S.A.) 91:5456; Fischer *et al.*, 1995, *Proc. Natl. Acad. Sci.* (U.S.A.) 92:5331; Lohmann *et al.*, 1995, *Biotechniques* 18:200; Reeves *et al.*, 1995, *Biotechniques* 18:18; and Maser *et al.*, 1995, *Semin. Nephrol.* 15:29, each of which is incorporated herein by reference.

The subtracted tester cDNA pool and a separate cDNA pool prepared in the same way from a cell line or tissue that does not express (or expresses at lower levels) the rare protein is amplified with suitable arbitrary primer(s) (i.e., primers having a predetermined sequence that is selected without reference to a sequence of a desired differentially expressed mRNA) for a suitable number of amplification cycles to generate sufficient amplification product for display and recovery of desired species, as can be determined empirically. The primer(s) may comprise 5'-terminal sequences which serve to anchor other PCR primers (distal primers) and/or which comprise a restriction site or half-site or other ligatable end. The amplified products are usually labeled and are typically resolved by electrophoresis on a polyacrylamide gel; the location(s) where label is present in the subtracted tester cDNA but not present (or present at much lower levels) in the control cDNA are excised, and the labeled product(s) is (are) recovered from the gel portion, typically by elution.

The resultant recovered product species (typically an expressed sequence tag or EST cDNA) can be subcloned into a replicable vector with or without attachment of linkers, amplified further, and/or sequenced directly. Once the EST(s) is recovered, it can be used to obtain a substantially full length cDNA from a cDNA library. The EST(s) can be sequenced and the sequence information used to generate a primer for primer extension (5'-RACE), or the EST can be labeled and used as a hybridization probe to identify larger cDNA clones from a cDNA library. Genomic or full length cDNA clones corresponding to ESTs can be isolated from clone libraries (e.g., available from Clontech, Palo Alto, CA) using the labeled EST (e.g., by nick-translation or end-labeling) or other hybridization probes with nucleotide sequences corresponding to those identified in the EST in conventional hybridization screening methods.

Thus, double stranded cDNA is made from total RNA purified by CsCl gradient centrifugation. In general, mix 5 μg of total RNA, 0.5 μg oligo dT (12 to 18 bases), and water (deionized water is routinely used) in a total of 7 μl, denature RNA at 95 degrees C for 5 to 10 minutes, and placed on ice. The denatured RNA and oligo dT is then added to a tube containing 4 μl of 5x first strand synthesis buffer (BRL), 2 μl of 0.1 M DTT (BRL), 1 μl of dNTP (10 mM each), and 1 μl of RNAsin (Pharmacia), and warmed for 2 minutes at 42 degrees C. About 5 μl of Superscript II™ reverse transcriptase (BRL) is added to the reaction mixture, and first strand cDNA synthesis is performed at 42 degrees C for 60 minutes. Then, the reaction mixture is placed on ice and is ready for the synthesis of second strand. The first strand cDNA is added to a tube containing 111.1 μl of water, 16 μl of 10x *E. coli* DNA ligase buffer, 3 μl of dNTP (10 mM each), 1.5 μl of *E. coli* DNA ligase (15 units, BRL), 7.7 μl *E. coli* DNA polymerase (40 units, Pharmacia), and 0.7 μl of *E.* coli RNAse H (BRL). The reaction mixture is incubated for two hours at 16 degrees C, and then 1 μl of T4 DNA polymerase (10 units, Pharmacia) is added. The incubation continues for 5 more minutes at the same temperature, and the reaction is stopped by the addition of 2 μl of 0.5 M EDTA and phenol/chloroform extraction, usually performed twice. The double-stranded cDNA is precipitated with ethanol and resuspended in 12 μl of TE buffer.

The cDNA is then modified by the addition of linkers. Mix 10 μl of cDNA prepared as above with 4 μl of 10x buffer for *RsaI*, 21 μl of water, and 5 μl of *RsaI* (25-50 units), and incubate the mixture for two hours at 37 degrees C. Four μl is removed and checked on an agarose gel (1%) along with the uncut cDNA for completion of digestion. The restriction enzyme is then inactivated for 10 min. at 65 degrees C.

The linkers are prepared as double stranded oligonucleotides by mixing 10 μg of each of:

NotA (5'-pATAGCGGCCGCAAGAATTCA-NH_2-3'); and

NotB (5'-TGAATTCTTGCGGCCGCTAT-3'); or

Ancol (5'-pCAGAAGCTTGGTTGGATCCAGCAAG-NH_2-3'); and

PCR02 (5'CTTGCTGGATCCAACCAAGCTTCTG-3'),

with 5.6 μl 10x buffer (One for all™, Pharmacia) and water to a final volume of 56 μl. Heat the mixture at 68 degrees C for 5 minutes, then 55 degrees C for 5 minutes, and then 45 degrees C for 10 minutes. Add 55 μl of double stranded oligonucleotide NotAB to the tube containing the digested tester cDNA (HUVEC, BJ, IMR90, IDH4, 293 or testes tissue – the telomerase negative cell lines are used as controls). Add 55 μl of double stranded oligonucleotide Ancol-PCR02 to the

tube containing the digested driver cDNA (HUVEC). To both tubes add 2 µl of 100 mM ATP, 3.3 µl of 10x lipase buffer (Pharmacia), 1 µl of T4 DNA ligase (Pharmacia), and water to 100 µl. The reaction mixture is incubated at 15 degrees C overnight. The reaction mixture is then removed from a 15 degrees C water bath to room temperature and incubated for another two hours. The ligated cDNA is extracted with phenol/chloroform twice and ethanol precipitated. The pellet is resuspended in 12 µl of TE buffer. Half of the product is loaded on a 1.4% low melting point agarose gel, and DNA with a size range from 100 to 1600 base pairs is excised.

PCR amplification of the tester and driver cDNA libraries is carried out by taking about 1 µl of each gel slice isolated as above (melted at 65 degrees C before use) and mixing with 10 µl of NotB (for testers – this oligonucleotide serves as both the 5' and 3' primers) or PCR02 (for the driver), 5 µl of 10x PCR buffer, 6 µl dNTP (2.5 mM each), 1 unit of *Taq* polymerase (Boehringer Mannheim or Perkin Elmer), 1 unit of *Pfu* polymerase (Stratagene), 0.2 µg of gene 32 protein (Boehringer Mannheim), and water to 50 µl. PCR is performed for 20 cycles at 94 degrees C for 45 sec., 60 degrees C for 45 sec., and 72 degrees C for 2 min., with a 5 min. extension at 72 degrees C after completion of the last cycle. The driver is PCR amplified in multiple reactions to make enough DNA for photobiotinylation.

Photobiotinylation of the driver cDNA is conveniently accomplished as follows. About 100 µg of driver cDNA in 1 mM EDTA is mixed with 100 µl of photo biotin (Vector). This mixture is placed on ice with the lid open and irradiated for 15 min. with a light source located about 10 cm away from the tube. After the irradiation, 30 µl of 1 M Tris-Cl (pH 9.1) is added to the tube, and the biotinylated DNA is extracted with water-saturated butanol several times (4X) until the orange color disappears from the aqueous phase. The extraction process is repeated once, and the biotinylated DNA is precipitated with ethanol and resuspended in TE buffer to a final concentration of 1 µg/µl.

Subtraction hybridization is conveniently accomplished as follows. Mix 8 µg of biotinylated driver DNA with 0.4 µg of tester DNA (concentrations estimated by OD measurement and ethidium bromide staining of the gel). The mixed DNA is precipitated with ethanol and resuspended in 10 µl of HE buffer (10 mM HEPES, pH 7.3, 1 mM EDTA). The DNA is denatured at 100 degrees C for 4 min. and transferred to ice. About 10 µl of 2X hybridization solution containing 1.5 M NaCl, 50 mM HEPES, pH 7.3, 10 mM EDTA, and 0.2% SDS is then added to the tube. Two drops of mineral oil are added, and the DNA is denatured again at 100 degrees C for 4 min. and transferred to a water bath at 68 degrees C. The hybridization is performed at this temperature for 22 hours. Biotinylated DNA is

removed with streptavidin MagneSphere™ Paramagnetic Particles (Promega), and the tester DNA remaining is recovered.

A second subtraction is performed by mixing recovered tester DNA (about 80 µl) with 8 µl (8 µg) of biotinylated driver DNA and then precipitation with ethanol. The precipitated DNA pellet is resuspended in 10 µl of HE buffer. The denaturation, hybridization, and recovery are performed as above; however, the second hybridization is performed for only 2 hours at 68 degrees C. PCR amplify the recovered DNA (0.3 µl) for 18 cycles in a reaction mixture containing 2 µl of 10X *Pfu* polymerase buffer, 2.5 µl of 2.5 mM dNTP, 0.2 µl of *Taq* polymerase (1 unit), 0.4 µl of *Pfu* polymerase (1 unit), 0.04 µl of T4 gene 32 protein, and water to 20 µl. The products are checked on a 1% agarose gel to confirm relative concentrations. The subtraction hybridization can be repeated on these samples. The final subtracted samples are PCR amplified (18 cycles) and diluted (1 to 10 or 1 to 15) and used for enhanced differential display.

Enhanced differential display of subtracted cDNA involves PCR amplification with 5' arbitrary primer(s) and a 3' oligo dT primer with two randomized bases at the 3' end, recovery of bands identified as containing cDNA corresponding to differentially expressed mRNAs, and PCR amplification, sequencing, and/or cloning of the bands identified. Add 1 µl of one 5' primer (20 µM stock) or two 5' primers (half of each) or 1.2 µl of one 5' primer (1 µl) and one 3' primer (0.2 µl) to the tube. Add 1 µl of subtracted DNA to the same tube. To this mixture, add 8 µl of cocktail mix containing 1 µl of 10X PCR buffer for *Pfu* polymerase (commercially available), 1 µl of dNTP (2.5 mM each), 0.3 mM alpha-^{32}P-dATP, 0.1 µl of *Taq* polymerase, 0.2 µl of *Pfu* polymerase (Stratagene), 0.02 µl of T4 gene 32 protein (Boehringer Mannheim), and 5.38 µl water. Overlay one drop of mineral oil, and PCR amplify for 4 cycles at 94 degrees C for 45 sec., 39 degrees C for 1 min., and 72 degrees C for 1 min., and then 22 cycles at 94 degrees C for 45 sec., 60 degrees C for 1 min., and 72 degrees C for 1 min., with a final extension for 5 min. at 72 degrees C. About 5 µl of formamide/dye is added to the PCR product, and the products are denatured at 95 degrees C for 2-3 min. and loaded onto a prewarmed 6% polyacrylamide sequencing gel, which is run at 1900 to 2000 constant voltage (do not allow current to reach 50 mA) until the xylene cyanol dye is one inch from the bottom of the gel. The gel is dried under vacuum at 80 degrees C for 45 min. and exposed to PhosphorImager™ screen (for notebook record) and/or then to X-ray film at room temperature for one or two days (tape the gel to the film and punch three holes at the three corner of the gel and film for easy identification of bands).

Differentially expressed gene fragments appear as bands on the screen or film that are present in the lanes on the gel corresponding to the cDNA of the tester cells but present at lower levels or absent from the lanes corresponding to the cDNA of the control lanes. The bands can be recovered from the gel by first aligning the gel with the film or screen (based on the three holes and marks) and then excising the bands of interest with a razor blade and transferring the gel slice to an Eppendorf™ tube. Rinse the razor blade between each cutting operation to avoid cross contamination. To remove the urea and paper backing used with sequencing gels without substantial loss of the desired DNA, add about 900 µl of TE buffer to the tube containing the gel slice, incubate the tube at room temperature for 10 min., and then remove and discard the paper and TE buffer. To prepare a solution of the desired DNA from the gel slice, the gel slice is suspended in 40 µl of TE buffer containing 100 mM NaCl and heated for 10 min. at 95-98 degrees C. The liquid is collected (a short centrifugation collects the liquid at the bottom of the tube) and serves as a source of the desired DNA.

This DNA can be PCR-amplified by placing 1-3 µl of recovered DNA in a 50 µl total reaction volume in a reaction mixture containing 6 µl of total primer(s), 5 µl of 10x PCR buffer for *Pfu* polymerase, 6 µl of dNTP (2.5 mM each), 0.25 µl of *Taq* polymerase, 0.5 µl of *Pfu* polymerase, 0.05 µl of T4 gene 32 protein, and water. The PCR is performed for 25 cycles at 94 degrees C for 45 sec., 60 degrees C for 1 min., and 72 degrees C for 1 min., with a 5 min. extension at 72 degrees C at the end of the last cycle. The PCR products can be stored or further processed, i.e., subcloned and sequenced.

The availability of plasmids comprising restriction fragments corresponding to the open reading frames of the TPC2 and TPC3 genes makes possible the efficient isolation of these gene and gene products from other mammalian cells as well as the chemical synthesis in vitro of these genes and gene products and related reagents, i.e., peptides, oligonucleotides, antibodies, and antibody fragments.

C. RT-PCR Protocol for TPC3

Cell extracts are prepared using CHAPS, as described for the TRAP assay (TRAP-eze™ kit, Oncor). About 2 µl of cell extract are used per assay; typically 30 - 35 cycles of PCR are performed. Total RNA is prepared using the TRIzol™ RNA extraction method (Life Technologies) on cell pellets or CHAPS extracts. Each PCR tube contains: 15 µl of water; 2.5 µl of 25 µM $Mn(OAc)_2$; 5.5 µl of 5X EZ buffer (Perkin Elmer); 0.3 µl of 25 µM dNTPs; 1 µl of r*Tth* DNA polymerase buffer (Perkin Elmer); 0.1 µl (300 µM) of primer TF2 (5'-CTCACTGTAGACACTGCCTC-AGTTTC-3'); and 0.1 µl (300 µM) of primer TR2 (5'-CAGAGGCTGGCACTGGAA-

CTCAAGATC-3') in a total volume of ~25 µl. RT-PCR conditions include a six minute treatment at 94 degrees C to denature protein-RNA complexes; a thirty minute treatment at 65 degrees C for the reverse transcription reaction; a 1.5 minute treatment at 94 degrees C to denature DNA-RNA complexes; thirty cycles of PCR amplification with each cycle comprising a 30 second treatment at 94 degrees C and a 30 second treatment at 65 degrees C; and a final extension reaction by treatment for seven minutes at 60 degrees C. After PCR, the samples can be analyzed by gel electrophoresis using 1X TBE polyacrylamide gels and staining with SYBR-Green I. Tests showed that this primer set amplifies band of correct size in both mortal and immortal cell lines and demonstrate that the TPC3 mRNA is expressed more abundantly in immortal cell lines.

D. RT-PCR Protocol for hTR

First strand cDNA synthesis is performed by mixing total RNA (1 µg) with 40 to 80 ng random hexamer in 11 µl, heating to 95 degrees C for 5 min. to denature the nucleic acids (the thermal cycler may be used for this step), and then cooling on ice. The reaction mixture (8 µl) containing 4 µl of 5X buffer (BRL, provided with the RTase), 2 µl of 0.1 M DTT,1 µl of 10 mM dNTP (each), and 1 µl of RNAse inhibitor (Pharmacia) is added to the denatured RNA and hexamer mixture and placed in a water bath at 42 degrees C. After a 1-2 min. incubation, 1 µl of Superscript II™ RTase (BRL) is added to the mixture and the incubation continued for 60 min. at 42 degrees C. The reaction is stopped by heating the tube containing the reaction mixture for 10 min. at 95 degrees C. The first strand cDNA is collected by precipitation and brief centrifugation and aliquoted to new tubes, in which it can be quickly frozen on dry ice and stored at -80 degrees C, if necessary, for later use.

PCR amplification of hTR cDNA with specific primer sets can be generally accomplished as follows. About 1 µl of cDNA is used for each primer set. For a 10 µl PCR with ^{32}P-dATP nucleotide, 1 µl of cDNA is mixed with 1 µl of 10X *Taq* buffer, 20 pmol of each primer, 1 µl of 2.5 mM dNTP, 5 µCi alpha-^{32}P-dATP, 1 unit of *Taq* polymerase (Boehringer Mannheim), 1 unit of *Taq* antibody (Clontech), 0.2 µg of T4 gene 32 protein (Boehringer Mannheim), and water to 10 µl. One drop of mineral oil is then added to the tube. The conditions for PCR amplification for hTR are about 20 cycles of amplification, with each cycle comprising a treatment at 94 degrees C for 45 sec., 60 degrees C for 45 sec., and 72 degrees C for 1.5 min. The primers used for the RT-PCR of hTR are shown below.

Upstream primer: F3b, 5'-TCTAACCCTAACTGAGAAGGGCGTAG-3';
Downstream primer: R3c, 5'-GTTTGCTCTAGAATGAACGGTGGAAG-3'.

Amplification of hTR with the F3b and R3c primer pair produces a 126 bp product. PCR products labeled with ^{32}P can be conveniently detected by adding 5 µl of a formamide/dye mixture to the products, heating the products to denature the nucleic acids, separating the products by 6% urea polyacrylamide gel electrophoresis, and then exposing a PhosphorImager™ cassette or X-ray film to the gel.

The invention has been described in terms of preferred embodiments and illustrated by way of example and is claimed below.

WHAT IS CLAIMED IS:

1. A recombinant mammalian host cell containing:

(i) a recombinant or synthetic nucleic acid comprising at least 15 contiguous nucleotides corresponding to an open reading frame sequence of a human gene TPC2 contained in a human DNA insert of an ~3.5 kb *Not*I-*Bst*EII restriction fragment of plasmid pGRN109; or

a synthetic or recombinant peptide or protein comprising at least 10 contiguous amino acids corresponding to an amino acid sequence encoded by said open reading frame sequence; and

(ii) a recombinant or synthetic nucleic acid comprising at least 15 contiguous nucleotides corresponding to an open reading frame sequence of a human gene TPC3 contained in a human DNA insert of an ~1.4 kb *Eco*RI-*Bam*HI restriction fragment of plasmid pGRN92; or

a synthetic or recombinant peptide or protein comprising at least 10 contiguous amino acids corresponding to an amino acid sequence encoded by said open reading frame sequence of gene TPC3;

said TPC2 and TPC3 genes characterized in coding for proteins that regulate telomere length or modulate telomerase activity and are present in human or other mammalian cells that express telomerase activity.

2. The mammalian host cell of Claim 1 that further comprises a synthetic or recombinant nucleic acid comprising at least about 15 contiguous nucleotides corresponding to a contiguous nucleotide sequence of human hTR located in an ~2.5 kb *Hin*dIII-*Sac*I restriction fragment of pGRN33.

3. A recombinant or synthetic nucleic acid comprising at least about 15 contiguous nucleotides corresponding to a contiguous nucleotide sequence located in either:

(i) an open reading frame sequence of a human gene TPC2 contained in a human DNA insert of an ~3.5 kb *Not*I-*Bst*EII restriction fragment of plasmid pGRN109; or

(ii) an open reading frame sequence of a human gene TPC3 contained in a human DNA insert of an ~1.4 kb *Eco*RI-*Bam*HI restriction fragment of plasmid pGRN92;

said TPC2 and TPC3 genes characterized in coding for proteins that regulate telomere length or modulate telomerase activity and are present in human or other mammalian cells that express telomerase activity.

4. The nucleic acid of claim 3 that comprises at least 15 contiguous nucleotides corresponding to a contiguous nucleotide sequence of human DNA located in the ~3.5 kb *Not*I-*Bst*EII restriction fragment of plasmid pGRN109.

5. The nucleic acid of claim 3 that comprises at least 15 contiguous nucleotides corresponding to a contiguous nucleotide sequence of human DNA located in the ~1.4 kb *Eco*RI-*Bam*HI restriction fragment of plasmid pGRN92.

6. The nucleic acid of claim 4 that is an oligonucleotide probe no more than 50 nucleotides in length.

7. The nucleic acid of claim 5 that is an oligonucleotide probe no more than 50 nucleotides in length.

8. The nucleic acid of claim 4 that is a recombinant expression vector.

9. The nucleic acid of claim 5 that is a recombinant expression vector.

10. The recombinant expression vector of claim 8 that is selected from the group consisting of pGRN106 and pGRN109.

11. The recombinant expression vector of claim 9 that is selected from the group consisting of pGRN92, pGRN103, and pGRN104.

12. A recombinant host cell comprising a vector of claim 8.

13. A recombinant host cell comprising a vector of claim 9.

14. The recombinant host cell of claim 12 that is E. coli/pGRN109.

15. The recombinant host cell of claim 13 that is E. coli/pGRN92.

16. A purified or synthetic or recombinant peptide or protein comprising at least 10 contiguous amino acids corresponding to an amino acid sequence encoded by an open reading frame sequence of a human gene located in a human DNA insert in either:

(i) an ~3.5 kb *Not*I-*Bst*EII restriction fragment of plasmid pGRN109; or

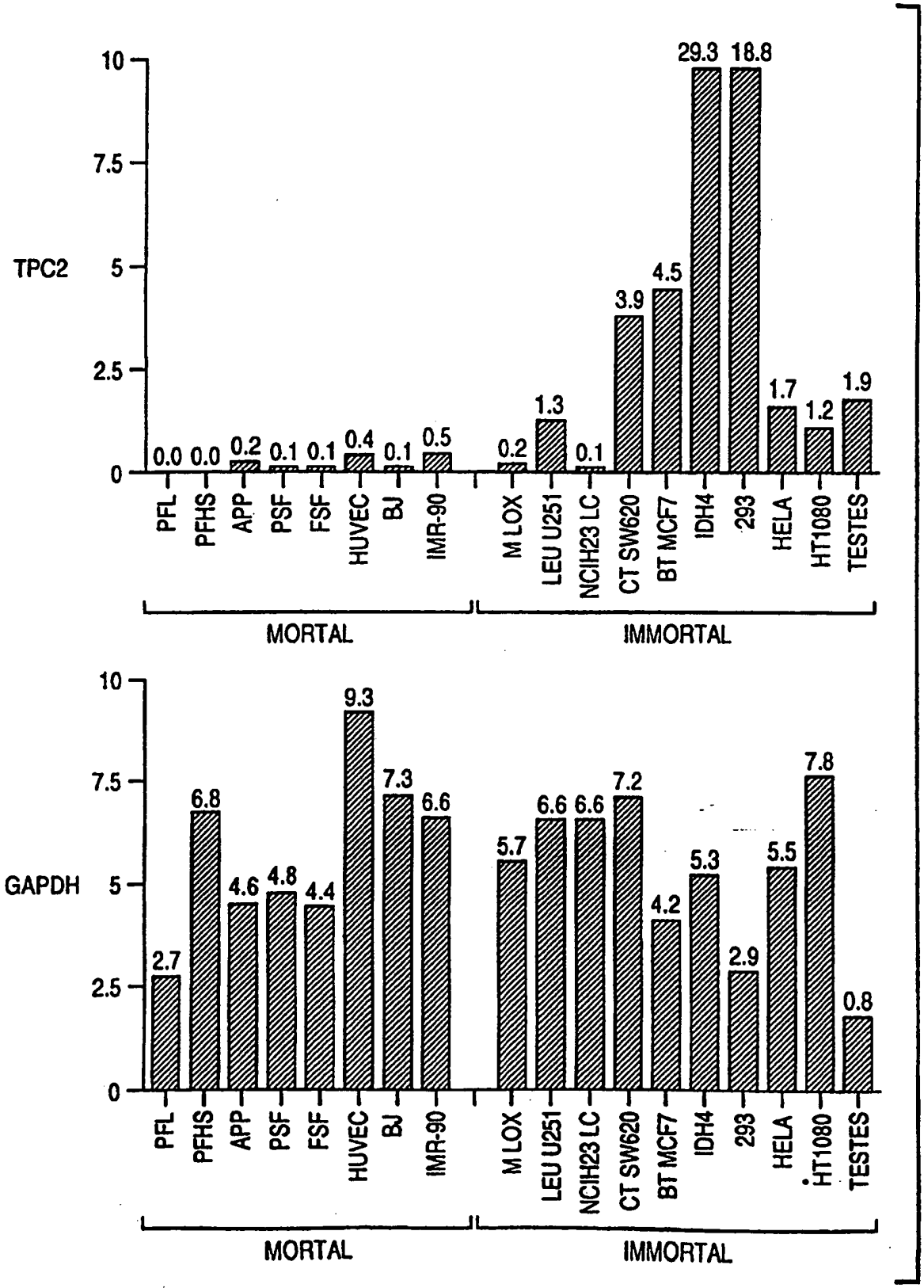
(ii) an ~1.4 kb *Eco*RI-*Bam*HI restriction fragment of plasmid pGRN92.

17. The protein of claim 16 that is purified or recombinant TPC2 protein.

18. The protein of claim 16 that is purified or recombinant TPC3 protein.

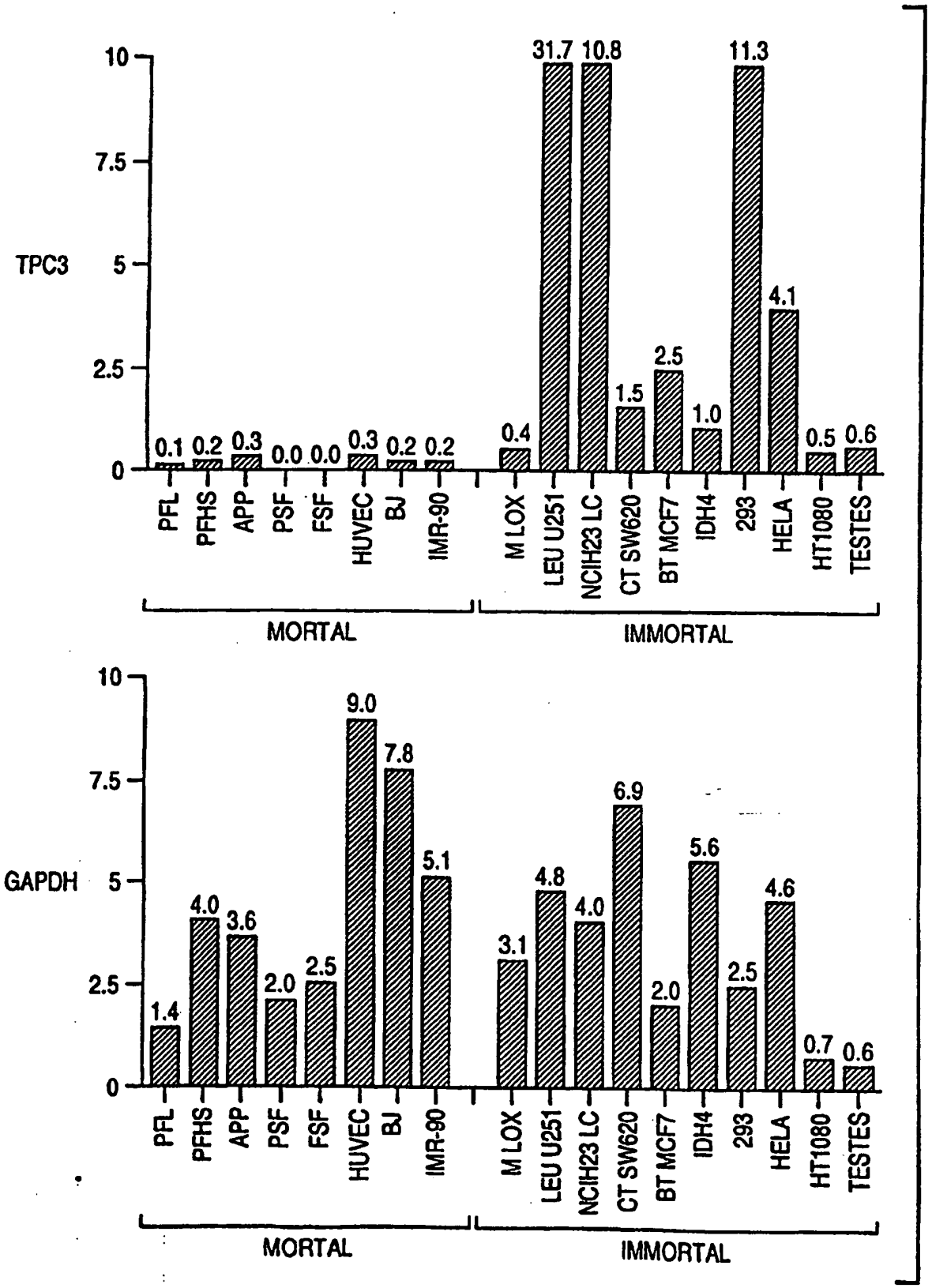
19. The peptide of claim 16 that is a fusion protein.

20. An antibody that specifically binds to an epitope on a peptide or protein of claim 16.



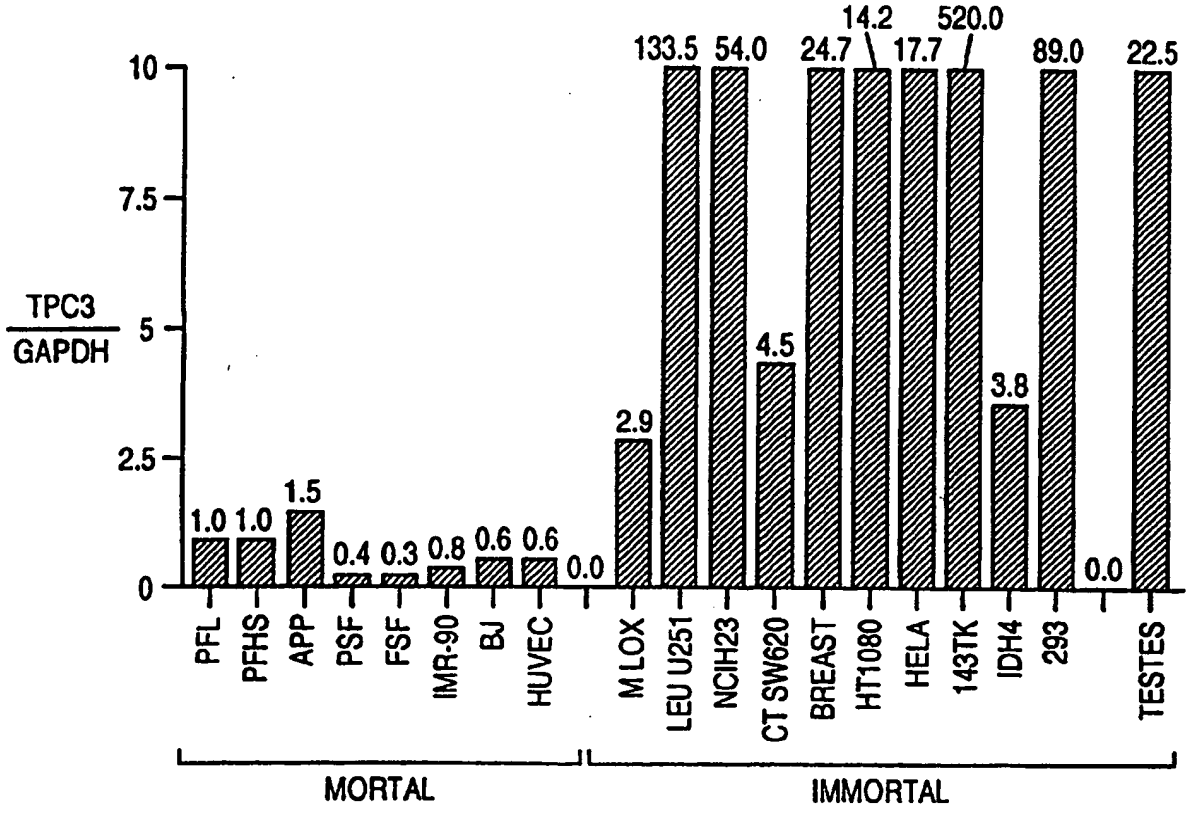
TPC2 and GAPDH mRNA

FIG. 1A



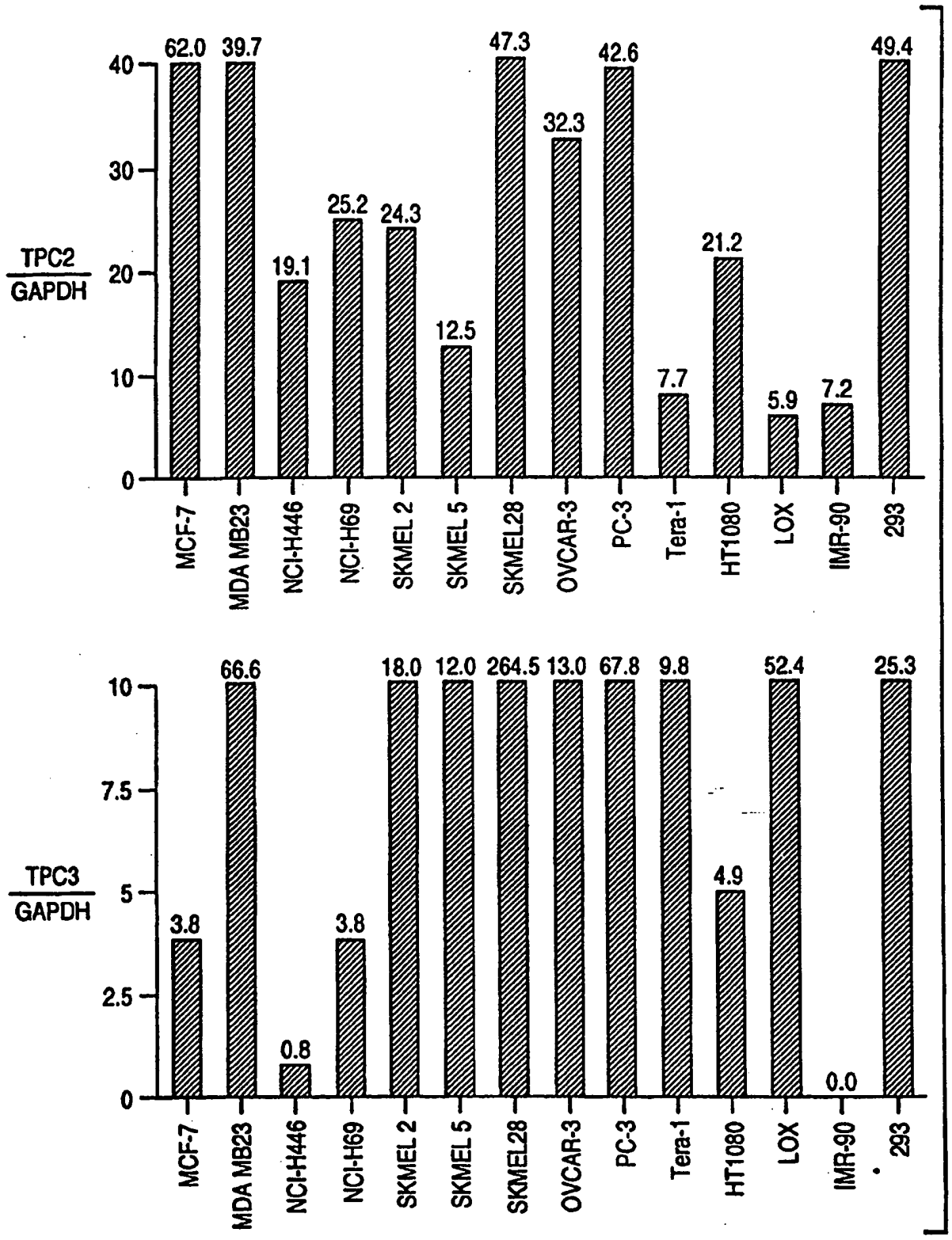
TPC3 and GAPDH mRNA

FIG. 1B



TPC3 normalized to GAPDH

FIG. 1C



TPC2 and TPC3 mRNA Levels

FIG. 2A

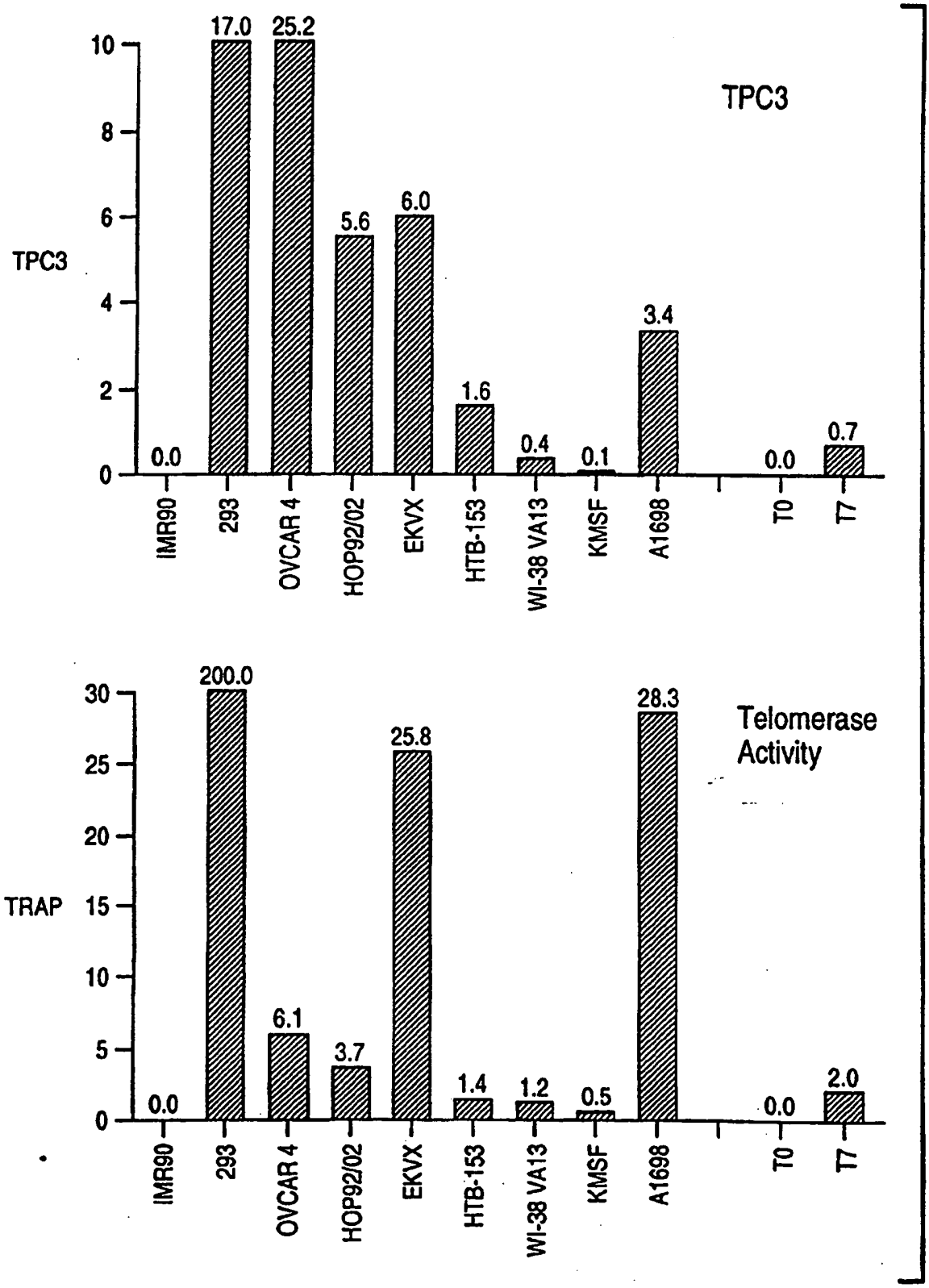

TPC3
TPC3
10
8
6
4
2
0
17.0
25.2
5.6
6.0
1.6
0.4
0.1
3.4
0.0
0.7
0.0
IMR90
293
OVCAR 4
HOP92/02
EKVX
HTB-153
WI-38 VA13
KMSF
A1698
T0
T7
Telomerase Activity
TRAP
30
25
20
15
10
5
0
200.0
6.1
3.7
25.8
1.4
1.2
0.5
28.3
0.0
2.0
IMR90
293
OVCAR 4
HOP92/02
EKVX
HTB-153
WI-38 VA13
KMSF
A1698
T0
T7

FIG. 2B

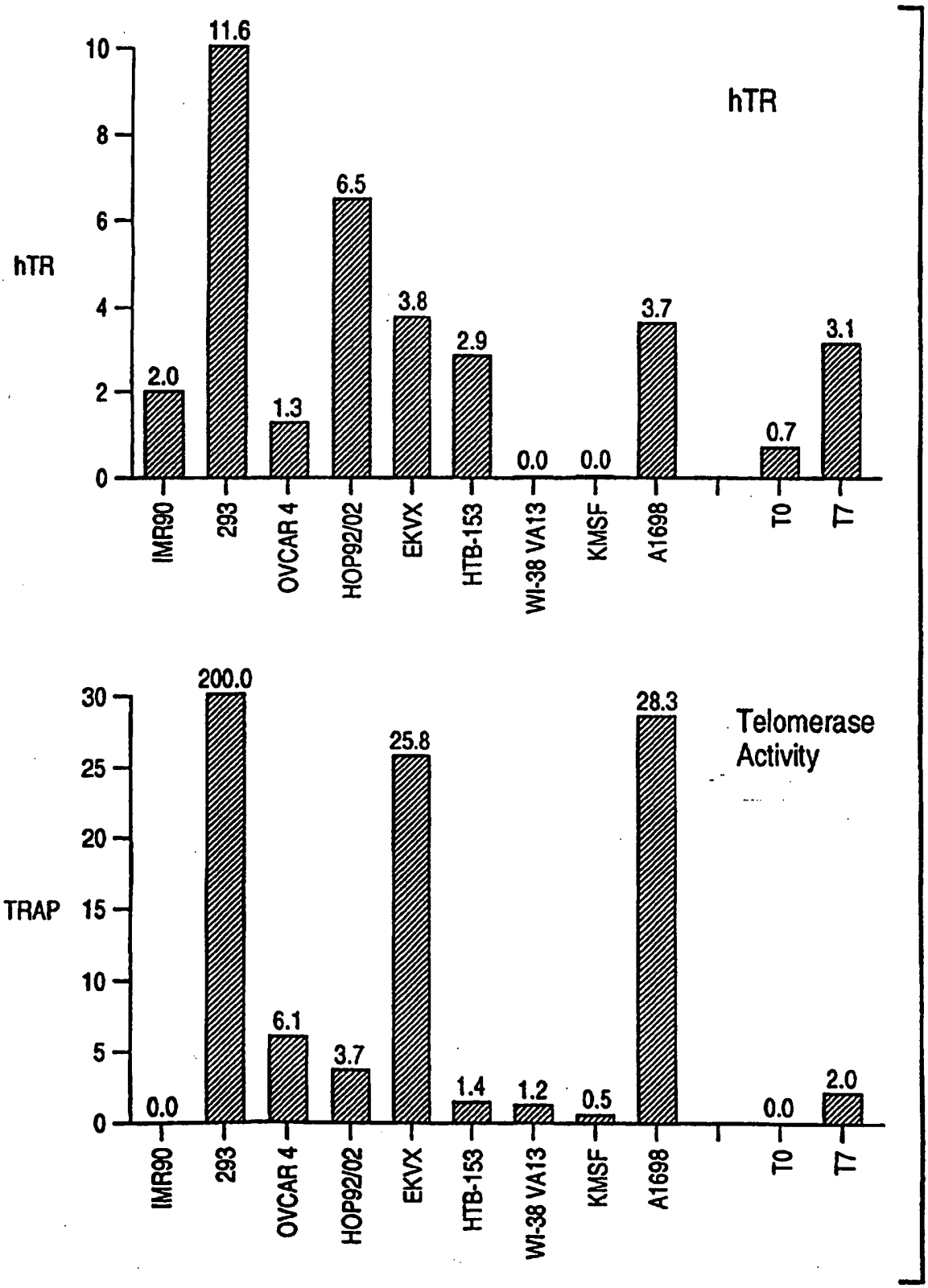

hTR
hTR
11.6
6.5
3.8
2.9
2.0
1.3
0.0
0.0
3.7
0.7
3.1
IMR90
293
OVCAR 4
HOP92/02
EKVX
HTB-153
WI-38 VA13
KMSF
A1698
T0
T7
Telomerase Activity
TRAP
200.0
28.3
25.8
6.1
3.7
1.4
1.2
0.5
0.0
2.0
IMR90
293
OVCAR 4
HOP92/02
EKVX
HTB-153
WI-38 VA13
KMSF
A1698
T0
T7

FIG. 2C

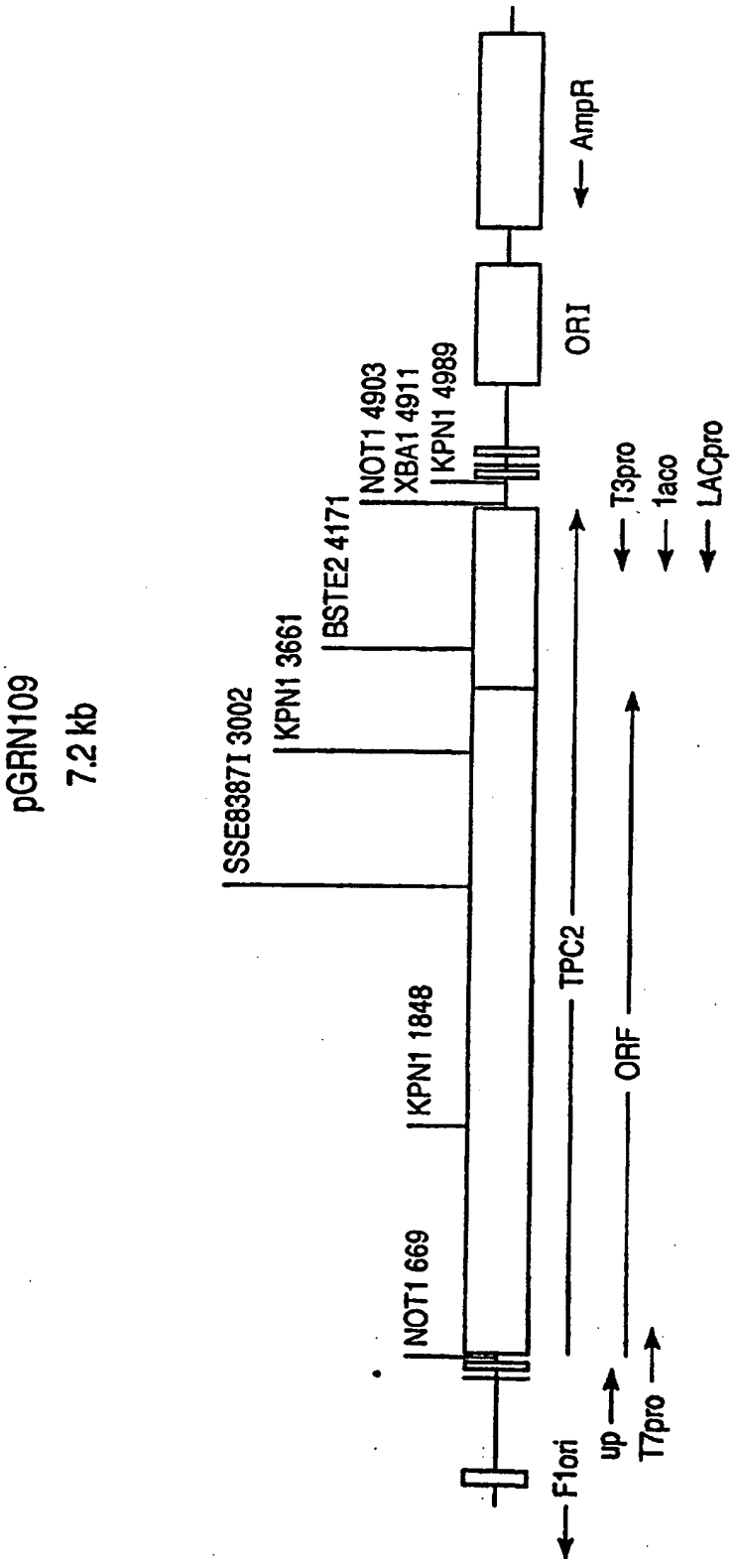

pGRN109
7.2 kb
AmpR
ORI
NOT1 4903
XBA1 4911
KPN1 4989
BSTE2 4171
KPN1 3661
SSE8387I 3002
KPN1 1848
NOT1 669
T3pro
1aco
LACpro
TPC2
ORF
F1ori
up
T7pro

FIG. 3

FIG. 4A

DNA and Translated Amino Acid Sequence of TPC2

```
    ProArgSerAlaAsnMetAlaAlaAlaThrValGlyArgAspThrLeuProGluHisTrp
  1 CCGCGCTCGGCGAACATGGCGGCGGCGACGGTCGGGCGGGACACTTTACCTGAGCATTGG

    SerTyrGlyValCysArgAspGlyArgValPhePheIleAsnAspGlnLeuArgCysThr
 61 TCCTACGGGGTGTGCCGGGATGGCCGCGTCTTCTTCATCAATGACCAGCTCCGCTGCACG

    ThrTrpLeuHisProArgThrGlyGluProValAsnSerGlyHisMetIleArgSerAsp
121 ACCTGGCTGCACCCGCGCACCGGGGAGCCCGTCAACTCGGGCCACATGATCCGCTCAGAC

    LeuProArgGlyTrpGluGluGlyPheThrGluGluGlyAlaSerTyrPheIleAspHis
181 CTGCCCCGCGGCTGGGAGGAGGGCTTCACGGAGGAGGGCGCCAGCTACTTCATCGACCAT

    AsnGlnGlnThrThrAlaPheArgHisProValThrGlyGlnPheSerProGluAsnSer
241 AACCAGCAGACCACAGCATTCAGGCATCCTGTGACGGGACAGTTTTCTCCAGAAAATAGT

    GluPheIleLeuGlnGluGluProAsnProHisMetSerLysGlnAspArgAsnGlnArg
301 GAATTCATTCTTCAAGAAGAGCCGAATCCACATATGTCGAAGCAAGACAGAAACCAAAGA

    ProSerSerMetValSerGluThrSerThrAlaGlyThrAlaSerThrLeuGluAlaLys
361 CCGTCCAGCATGGTCAGTGAAACATCCACGGCTGGGACCGCCTCCACCCTGGAGGCCAAG

    ProGlyProLysIleIleLysSerSerSerLysValHisSerPheGlyLysArgAspGln
421 CCTGGACCCAAGATCATAAAGTCCAGCAGTAAAGTCCACAGCTTTGGGAAGAGAGACCAG
```

FIG. 4B

DNA and Translated Amino Acid Sequence of TPC2

```
     AlaIleArgArgAsnProAsnValProValValValArgGlyTrpLeuHisLysGlnAsp
481  GCCATTCGGAGGAACCCCAATGTTCCCGTGGTGGTGAGGGGCTGGCTGCACAAGCAGGAC

     Ser  GlyMetArgLeuTrpLysArgArgTrpPheValLeuAlaAspTyrCysLeuPhe
541  AGTTYTGGGATGAGGCTGTGGAAAAGGAGGTGGTTTGTGCTTGCTGATTACTGCTTATTT

     TyrTyrLysAlaGluLysLysArgSerSer  SerIleProLeuPro  TyrVal
601  TACTATAAAGCCGAGAAGAAGCGGTCCTCGXGGAGCATCCCCTTGCCCAG3TACGTGAT3

     SerProValAlaProGluAspArgIleSerArgLysTyrSerPheLysAlaValHisThr
661  TCTCCTGTGGCCCCTGAGGATCGCATAAGCCGCAAATATTCCTTTAAGGCTGTGCACACG

     GlyMetArgAlaLeuIleTyrAsnSerSerThrAlaGlySerGlnAlaGluGlnSerGly
721  GGGATGCGAGCGCTCATCTATAACAGCTCCACAGCGGGCTCTCAGGCCGAGCAGTCAGGC

     MetArgThrTyrTyrPheSerAlaAspThrGlnGluAspMetAsnAlaTrpValArgAla
781  ATGAGGACCTACTACTTCAGTGCCGACACCCAGGAGGACATGAACGCTTGGGTCAGGGCC

     MetAsnGlnAlaAlaGlnValLeuSerArgSerSerLeuLysArgAspMetGluLysVal
841  ATGAACCAGGCTGCACAGGTGCTGTCTCGATCGTCACTGAAGAGGGATATGGAGAAGGTG

     GluArgGlnAlaValProGlnAlaAsnHisThrGluSerCysHisGluCysGlyArgVal
901  GAGCGGCAGGCTGTCCCCCAGGCCAACCACACAGAGTCCTGTCACGAATGTGGCCGGGTG
```

FIG. 4C

DNA and Translated Amino Acid Sequence of TPC2

```
     GlyProGlyHisThrArgAspCysProHisArgGlyHisAspAspIleValAsnPheGlu
 961 GGACCCGGACATACGAGAGATTGTCCTCATCGTGGCCATGATGACATTGTCAACTTCGAG

     ArgGlnGluGlnGluGlyGluGlnTyrArgSerGlnArgAspProLeuGluGlyLysArg
1021 AGGCAGGAGCAGGAGGGAGAGCAGTACCGTTCCCAGAGGGACCCACTGGAGGGCAAGCGG

     AspArgSerLysAlaArgSerProTyrSerProAlaGluGluAspAlaLeuPheMetAsp
1081 GACCGGAGCAAGGCCAGGTCTCCGTACTCGCCAGCCGAGGAGGATGCCTTGTTTATGGAT

       Pro   GlyProArg   GlnGlnAlaGlnProGlnArgAla   LysAsnGlyMet
1141 T4ACCCAYTGGCCCAAGAG0CCAGCAGGCACAGCCCCAACGGGCAGA6AARAATGGAATG

     LeuPro       TyrGlyProGly       AsnGly   GlyGly   GlnArg   Phe
1201 CTGCCTGCYT3ATATGGCCCAGGA6AACA6AATGGGA3TGGTGGGT5CCAGCGGGCYTTT

       ProArgThrAsn   GluLysHisSerGlnArgLys   AsnLeuAlaGlnValGlu
1261 CYTCCCAGGACCAACCYTGAAAAACACAGCCAAAGGAAGA6CAATCTGGCCCAGGTGGAG

     HisTrpAlaArgAlaGlnLysGlyAspSerArgSerLeuProLeuAspGlnThr
1321 CACTGGGCAAGGGCCCAGAAAGGGGATAGCAGGAGTCTTCCCTTGGACCAGACG3TTC3T

     ArgGlnGly   GlyGlnSerLeu   PheProGluAsnTyrGln       ProLysSer
1381 CGCCAGGGTC3TGGCCAATCCCTGTC3TTCCCAGAAAACTACCAGAYTYTTCCCAAGAGC
```

FIG. 4D

DNA and Translated Amino Acid Sequence of TPC2

```
     ThrArgHisProSerGly      SerPro   ProArgAsnLeuProSerAspTyrLys
1441 ACCCGACACCCCTCGGGGGGYTCYTCGCCACYTCCCCGAAACCTGCCAAGTGACTACAAG

     TyrAlaGlnAspArgAlaSerHisLeu  MetSerSerGlu  Arg  GlyAlaPro
1501 TATGCGCAGGACCGAGCCAGCCACCTGAA6ATGTCGAGTGAA6A6CGCCGXGGCGCACCG

     GlyTrpHisArgValAla   Leu      AlaAlaAlaProAlaValProAlaArgGln
1561 GGATGGCACCGTGTGGCAGYTCTAC6A6TGGCAGCAGCGCCAGCAGTTCCGGCACGGCAG

     ProHisSerAlaHisLeuProTrpLeuPro   ValHisArgProGlyProGluGlnGlu
1621 CCCCACAGCGCCCATCTGCCTTGGCTCCCCA6AGTTCACCGACCAGGGCCGGAGCAGGAG

     His      GlyAlaProLeuHis   CysAlaSer   Ser   GlyHis   SerPro
1681 CATG3TA6AGGTGCCCCGCTCCATYT3TGTGCCTCCAT3TCCYT3GGACATCC3TCCCCC

     ArgThrProLysGly   ProThrProAlaAlaThrHisThrSerArg   Ser   Ser
1741 AGGACCCCCAAGGGTYTTCCCACCCCGGCGGCCACACACACCAGCAGA6CGAGTC5CAGT

     GluAlaThrGlyPro   Glu   CysGlyHisLeuProGlyGlyPheSerMetGlyTyr
1801 GAAGCCACCGGACCA6ARGARGA6TGTGGACATCTCCCTGGGGGATTCTCCATGGGTTAC

     Met   HisThrValSerAlaProSerLeuHisGlyLysSerAlaAspAspThrTyrLeu
1861 ATGAJCCACACCGTCAGCGCTCCCAGTTTACATGGAAAATCGGCTGATGATACCTACCTC
```

FIG. 4E

DNA and Translated Amino Acid Sequence of TPC2

```
     GlnLeu   Lys   Leu   TyrLeuAspLeuLysMetThrGlyArgAspLeuLeuLys
1921 CAGCTGAA6AAARA7CTGGA6TACCTGGATCTAAAGATGACAGGCCGGGACCTTCTCAAG

     AspArgSerLeuLysProValLysIleAlaGluSerAspThrAspValLysLeuSerIle
1981 GATCGAAGTCTGAAGCCTGTGAAGATCGCTGAGAGCGACACTGACGTCAAACTGAGCATC

     PheCysGluGlnAspArgValLeuGlnAspLeuGluAspLysIleArgAlaLeuLysGlu
2041 TTCTGTGAACAAGACAGGGTCCTCCAGGACTTGGAAGACAAGATACGAGCCCTTAAAGAG

     AsnLysAspGlnLeuGluSerValLeuGluValLeuHisArgGlnMetGluGlnTyrArg
2101 AACAAAGACCAGCTAGAATCTGTGCTGGAGGTGTTGCACAGACAGATGGAGCAGTACCGA

     AspGlnProGlnHisLeuGluLysIleAlaTyrGlnGlnLysLeuLeuGlnGluAspLeu
2161 GACCAGCCCCAGCACTTGGAGAAGATTGCCTACCAGCAGAAGTTGCTGCAGGAGGACCTT

     ValHisIleArgAlaGluLeuSerArgGluSerThrGluMetGluAsnAlaTrpAsnGlu
2221 GTCCATATCCGAGCTGAGCTCTCCAGAGAGTCCACTGAGATGGAAAATGCTTGGAACGAA

     TyrLeuLysLeuGluAsnAspValGluGlnLeuLysGlnThrLeuGlnGluGlnHisArg
2281 TACCTGAAGTTGGAGAATGATGTGGAACAGCTGAAGCAGACCCTGCAGGAGCAACACAGA

     ArgAlaPhePhePheGlnGluLysSerGlnIleGlnLysAspLeuTrpArgIleGluAsp
2341 AGAGCCTTTTTTTTCCAGGAGAAATCGCAGATACAGAAAGATCTATGGAGAATTGAAGAT
```

FIG. 4F

DNA and Translated Amino Acid Sequence of TPC2

```
     ValThrAlaGlyLeuSerAlaAsnLysGluAsnPheArgIleLeuValGluSerValLys
2401 GTCACTGCAGGCCTGAGTGCAAATAAAGAGAACTTCAGAATTCTAGTGGAGTCAGTAAAA

     AsnProGluArgLysThrValProLeuPheProHisProProValProSerLeuSerThr
2461 AATCCGGAGAGAAAAACGGTGCCTTTGTTTCCTCACCCGCCTGTGCCTTCACTCTCAACT

     SerGluSerLysProProProGlnProSerProProThrSerProValArgThrProLeu
2521 TCTGAGAGCAAGCCGCCCCCACAGCCCAGTCCTCCCACCAGCCCTGTGCGGACCCCTCTG

     GluValArgLeuPheProGlnLeuGlnThrTyrValProTyrArgProHisProProGln
2581 GAGGTTCGACTCTTCCCCCAGCTGCAAACCTACGTGCCGTACCGACCTCACCCACCCCAG

     LeuArgLysValThrSerProLeuGlnSerProThrLysAlaLysProLysValGlnGlu
2641 CTGAGGAAAGTGACATCCCCCCTTCAGTCACCAACTAAGGCGAAGCCCAAAGTTCAGGAA

     AspGluAlaProProArgProProLeuProGluLeuTyrSerProGluAspGlnProPro
2701 GATGAAGCACCTCCCAGGCCCCCACTCCCCGAACTCTACAGCCCAGAGGACCAGCCCCCG

     AlaValProProLeuProArgGluAlaThrIleIleArgHisThrSerValArgGlyLeu
2761 GCTGTGCCGCCTCTGCCAAGAGAGGCCACCATCATCCGGCACACATCTGTGCGGGGCCTC

     LysArgGlnSerAspGluArgLysArgAspArgGluLeuGlyGlnCysValAsnGlyAsp
2821 AAGCGGCAGTCAGACGAGAGGAAGCGAGACCGGGAGCTGGGGCAGTGTGTGAATGGGGAT
```

FIG. 4G

DNA and Translated Amino Acid Sequence of TPC2

```
     SerArgValGluLeuArgSerTyrValSerGluProGluLeuAlaThrLeuSerGlyAsp
2881 TCCAGGGTGGAGCTGCGGTCGTATGTCAGTGAGCCTGAGCTGGCGACCCTCAGCGGGGAC

     MetAlaGlnProSerLeuGlyLeuValGlyProGluSerArgTyrGlnThrLeuProGly
2941 ATGGCCCAGCCCTCCCTAGGACTTGTGGGCCCTGAGAGCAGGTACCAGACGCTGCCAGGC

     ArgGlyLeuSerGlySerThrSerArgLeuGlnGlnSerSerThrIleAlaProTyrVal
3001 AGAGGGCTCTCAGGGTCCACGTCAAGGCTCCAGCAGTCGTCCACCATTGCTCCCTACGTC

     ThrLeuArgArgGlyLeuAsnAlaGluSerSerLysAlaThrPheProArgProLysSer
3061 ACACTCCGGAGGGGTCTCAATGCCGAAAGCAGCAAGGCGACCTTCCCTAGACCTAAGAGT

     AlaLeuGluArgLeuTyrSerGlyAspHisGlnArgGlyLysMetSerAlaGluGluGln
3121 GCCTTGGAGCGCCTGTACTCAGGGGATCACCAGCGAGGCAAGATGAGTGCAGAGGAGCAG

     LeuGluArgMetLysArgHisGlnLysAlaLeuValArgGluArgLysArgThrLeuGly
3181 CTGGAGCGCATGAAGCGACACCAGAAGGCCCTGGTCCGAGAGCGCAAGAGGACACTGGGC

     GlnGlyGluArgThrGlyLeuProSerSerArgTyrLeuSerArgProLeuProGlyAsp
3241 CAAGGGGAGAGGACGGGCCTGCCCTCATCTCGCTACCTCAGCCGGCCGCTCCCTGGAGAT

     LeuGlySerValCys---
3301 CTTGGCTCAGTATGTTAGGAGGGGCCAGGCAGCGGGGCAGGGACAGGGAGCCGAGTGCCC
```

FIG. 4H

DNA and Translated Amino Acid Sequence of TPC2

```
3361 CTCAGAGTCCCCCAAACACAAGCACATCACACCTCCCAGTGAGAGAGCTGTCCATTGACC
3421 TACATGGTTCAGAGAACACCCCACGGGGCTGTTTGTCCACGACCCAGGCTGGACGAATGC
3481 CTGGTCAGAGGGTGACCTGAACCAGAGCTGGAGTGAGGATCAAACAGGCCCAGGAGCCTG
3541 AGGAAATACCCAGTCAGTCCTCCCAGCCGCGATGGAGAGGGGCCTTTGCAGGCGTTCGGA
3601 ATCTCGGCTGAATTCAGGACCTGGGAATACAGGGTTCAGAGAGGAGAGGAAGATGGT
3661 GACATGATTTGGTTAGAAGCACAAGCAAACTGATCAGCCTCCCAGACCTGCCAGCAGATG
3721 CTGTGTGAGGGTGATGGAGCACGGGGGTCACACCCCTGCCCCAAGGGCCACTGGTCTCCCT
3781 GGGCTTGCAGTGCAGAGGCCTCAGGGTGTCTGGGATTGCTGGGGAGG9CTGTGCTGCCCC
3841 CTGGTGGCGCTTCCTGGCGCTGCGCCCTGTCCACAGTCACCTTAGGACCCTTTGGAAACA
3901 TTCCATTTGACTTTTCCCTGTTGYTTGAAATCCCATGTTTCCCTAAACCTCTAGCCTGAT
3961 TGTTCTTTCCCTAATTCATTGCACAAGCTCCTTTGCTTTAGTGTTACCGCTCATTGCCT
4021 CTCTAATCCTGCCTGATTGTGTTTACAGAAGCTTCTGATTTGCATTGAACATGCTCTAAC
4081 TGGCCTGTGCTACTTATTACCGGGCTTGTAATAGCGGTTCTTGTCTCCATAGCCTGTTGA
4141 GTGTTCCCAGATGTGACTCACCTTTCTGCTGCCCTCTTCATGCAGGCCTACTGACTCATA
4201 ATTCACTTGTCCGTCGACGCGGCCGCGAATTC
```

FIG. 4I

DNA and Translated Amino Acid Sequence of TPC2

Code	Meaning
Any codes not listed below matches A, C, G, or T (any base)	
1	A, C, G, or T (any base)
2	A, C, G, or T (any base)
3	probably Cytosine
4	probably Thymine
5	probably Adenine
6	probably Guanine
7	maybe Cytosine
8	maybe Thymine
9	maybe Adenine
0	maybe Guanine
A	Adenine
B	C if preceded (5'-3') by T; G if preceded by A
C	Cytosine
D	C or T if followed (5'-3') by AA; any base if followed by AG
E	C or T if followed (5'-3') by CT; any base if followed by CG
F	A or G if followed (5'-3') by CT; any base if followed by GA
G	Guanine
H	A, C, or G (not T)
I	A or G if preceded (5'-3') by TT; any base if preceded by CT
J	A or C
K	G or T
L	A or T

FIG. 4J

DNA and Translated Amino Acid Sequence of TPC2

M C or G
N A, C, G, or T (any base)
O A, C, G, or T (any base)
P A or G if preceded (5'-3') by AG; any base if preceded by CG
Q A, C, or T (not G)
R A or G
S C, G, or T (not A)
T Thymine
U A, C, G, or T (any base)
V C or T if preceded (5'-3') by AG; any base if preceded by TC
W A, G, or T (not C)
X A nucleotide that has been arbitrarily added to join reading frames.
Y C or T
Z C if followed (5'-3') by T; G if followed by A

Complementary codes:
1234567890ABCDEFGHIJKLMNOPQRSTUVWXYZ
1265430987TZGIPVCSDKJLMNOEWYHAAFQXRB

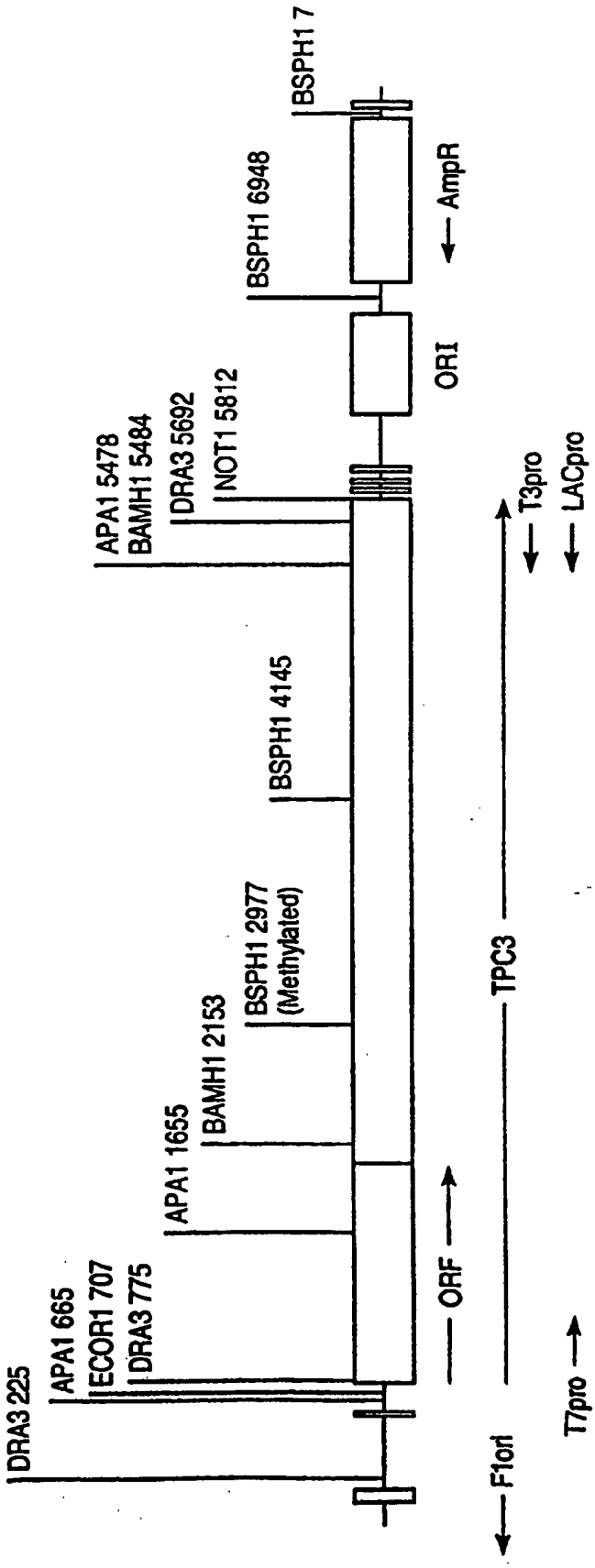

pGRN92
8.0 kb
BSPH1 7
BSPH1 6948
AmpR
ORI
APA1 5478
BAMH1 5484
DRA3 5692
NOT1 5812
T3pro
LACpro
BSPH1 4145
BSPH1 2977
(Methylated)
BAMH1 2153
APA1 1655
TPC3
ORF
DRA3 225
APA1 665
ECOR1 707
DRA3 775
T7pro
F1ori

FIG. 5

FIG. 6A

DNA and Translated Amino Acid Sequence of TPC3 ORF

```
   1 GGAAACGCAGTTTAAAACTCCAGCCCAGGCCCCGTCGCGCGTAGATGGCAGCGGAGGCGG

                                  ProAspProValGlnThrGlnLeuProProSerAlaProPhe
  61 CGGCGCGGGCCGGGGGTGACCAGATCCCGTTCAAACTCAGCTGCCACCAAGTGCGCCTTTT

     LeuSerGlyLeuArgPheCysThrAsnPheProValGluGlyGlySerAlaLeuSerGln
 121 CTCTCTGGATTGCGATTCTGCACGAATTTTCCAGTTGAGGGTGGTTCGGCGCTCAGCCAG
                                                              ***
     ProLeuProSerLysThrArgProTrpSerArgAsnLeuGlnAlaAspAlaAlaMetGln
 181 CCTCTGCCCTCGAAGACGCGGCCTTGGTCTAGGAACCTTCAGGCGGATGCCGCCATGCAG

     HisTyrGlyValAsnGlyTyrSerLeuHisAlaMetAsnSerLeuSerAlaMetTyrAsn
 241 CACTACGGGGTGAACGGCTACTCACTGCACGCCATGAACTCACTCAGCGCCATGTACAAC

     LeuHisGlnGlnAlaAlaGlnGlnAlaGlnHisAlaProAspTyrArgProSerValHis
 301 CTGCACCAGCAGGCAGCCCAGCAGGCCCAGCATGCCCCCGACTACCGGCCTTCAGTGCAT

     AlaLeuThrLeuAlaGluArgLeuAlaGlyCysThrPheGlnAspIleIleLeuGluAla
 361 GCGCTTACATTGGCTGAGCGCCTGGCTGGCTGTACATTTCAAGACATCATCTTGGAGGCC

     ArgTyrGlySerGlnHisArgLysGlnArgArgSerArgThrAlaPheThrAlaGlnGln
 421 CGTTATGGTTCCCAGCACCGCAAACAACGTCGCAGCCGCACAGCGTTCACGGCTCAGCAG

     LeuGluAlaLeuGluLysThrPheGlnLysThrHisTyrProAspValValMetArgGlu
 481 CTCGAGGCCCTGGAAAAGACCTTCCAGAAGACTCACTACCCAGATGTGGTGATGCGTGAG
```

FIG. 6B

DNA and Translated Amino Acid Sequence of TPC3 ORF

```
    ArgLeuAlaMetCysThrAsnLeuProGluAlaArgValGlnValTrpPheLysAsnArg
541 AGGCTGGCCATGTGCACCAACCTGCCTGAGGCCCGGGTGCAGGTGTGGTTCAAGAACCGC

    ArgAlaLysPheArgLysLysGlnArgSerLeuGlnLysGluGlnLeuGlnLysGlnLys
601 CGGGCCAAGTTCCGGAAGAAGCAGCGTAGCCTGCAGAAGGAACAGCTCCAGAAGCAGAAG

    GluAlaGluGlySerHisGlyGluGlyLysAlaGluAlaProThrProAspThrGlnLeu
661 GAGGCTGAGGGCTCCCATGGGGAAGGCAAGGCCGAGGCCCCCACTCCAGATACCCAGCTG

    AspThrGluGlnProProArgLeuProGlySerAspProProAlaGluLeuHisLeuSer
721 GACACTGAGCAGCCCCCACGTCTGCCTGGCAGCGACCCCCCTGCTGAGCTTCACCTGAGT

    LeuSerGluGlnSerAlaSerGluSerAlaProGluAspGlnProAspArgGluGluAsp
781 CTGTCTGAGCAGTCAGCCAGTGAGTCAGCCCCTGAGGATCAGCCGGACCGTGAGGAGGAC

    ProArgAlaGlyAlaGluAspProLysAlaGluLysSerProGlyAlaAspSerLysGly
841 CCCAGGGCAGGGGCTGAGGACCCCAAAGCTGAGAAGAGCCCTGGGGCTGACAGCAAGGGG

    LeuGlyCysLysArgGlySerProLysAlaAspSerProGlySerLeuThrIleThrPro
901 CTGGGCTGCAAGAGGGGCAGCCCCAAGGCAGATTCCCCAGGCAGCCTGACCATCACTCCT


    ValAlaProGlyGlyGlyLeuLeuGlyProSerHisSerTyrSerSerSerProLeuSer
961 GTGGCCCCAGGGGGTGGCCTCCTGGGCCCCTCCCACTCCTATTCCTCGTCCCCGCTGAGC
```

FIG. 6C

DNA and Translated Amino Acid Sequence of TPC3 ORF

```
     LeuPheArgLeuGlnGluGlnPheArgGlnHisMetAlaAlaThrAsnAsnLeuValHis
1021 CTCTTCCGTCTGCAGGAGCAATTCCGCCAGCATATGGCGGCCACCAACAACCTGGTGCAC

     TyrSerSerPheGluValGlyGlyProAlaProAlaAlaAlaAlaAlaAlaAlaAlaVal
1081 TACTCGTCCTTCGAAGTAGGGGGTCCGGCCCCTGCTGCTGCAGCGGCGGCTGCTGCTGTG

     ProTyrLeuGlyValAsnMetAlaProLeuGlySerLeuHisCysGlnSerTyrTyrGln
1141 CCCTACCTGGGCGTCAACATGGCCCCGCTGGGCTCACTGCACTGCCAGTCCTACTACCAG

     SerLeuSerAlaAlaAlaAlaAlaHisGlnGlyValTrpGlySerProLeuLeuProAla
1201 TCCCTGTCAGCAGCCGCTGCTGCCCACCAGGGTGTGTGGGGGTCTCCTCTGCTGCCTGCA

     ProProAlaGlyLeuAlaProAlaSerAlaThrLeuAsnSerLysThrThrSerIleGlu
1261 CCCCCAGCAGGCCTGGCTCCTGCATCAGCTACCCTGAACAGTAAAACCACAAGCATCGAG

     AsnLeuArgLeuArgAlaLysGlnHisAlaAlaSerLeuGlyLeuAspThrLeuProAsn
1321 AACCTGCGGCTCCGGGCCAAGCAGCACGCGGCCTCCCTGGGACTCGATACGCTGCCCAAC

     ---
1381 TGACTGTCTGGCTTCCAACCCAGCCAGGGGTCTTAGGTGTCCCCTCCTAGCCCTGTGGTT
1441 ATCCCTAGGTGGCTCTCGAGGAGTTAACTCCATGAGCCCAGGGATCCTAGGGCCTGGGGT
1501 CCTGTTCCCTGCTCCGCTTCCCCATACCCCAGCCCGAGGTGAAGCCCACACCTACACACC
1561 CTCTGCATGGCCCTGCCTGGACCCCATGGAGGCCGAATAGGGAGGAGGTGAGAGGCTGGG
1621 GTGCCCCAAGCTTCCCTCGGAGAAGTGAGAGGCTCTCCCTGGCTAGATCCTCATCTCAAT
```

FIG. 6D

DNA and Translated Amino Acid Sequence of TPC3 ORF

```
1681 AGCACCTCCTCCCTTTTCTCCCTATCCTTCTGCCCCCTAGTAAGTCTACGTGTGGAATGT
1741 GAGATATAAATATAAATATATAAAGCTATATTTTCAGGCTCCTGCCTGCCCCAGGCCCCC
1801 TGCCCCACTCCCATCTCTTCTTCCCTGCCACCCCTCCCTGCAGCCTCCGCGGCTCACTCC
1861 AGCCATCCCTTCTGTTTCTCCTTCTCTCTCCTTCCTTCTTCCCTTGATCTCCCTCTTCCT
1921 GTCTCTGTCCTGGTCCCTGCCCCCGTCTCGGCCCAGCCTCTGTATTCTCCACCCTTGATC
1981 TTTCTCCTTGTCTCTCCCGCTGCCCCTGGTTTCTTCCTTTGGTGTTGGCTGTGTTGGTAT
2041 CATCAGTTCTTGAGCTATATTTTGTTTGGGGTTGTGGCTGGTTTTGGTTTTAGTAATTTT
2101 GCGACTTCCCGTTGCTCTCCTTCTATTCCCTTCCTTCTGCCCTGCCTGCCTCCCTGCACC
2161 TGCGGCCTCTCTAGGAAGCTGTTCCTTTCTATGCCCAATAGAAGCAACAAGGCCCTAGCT
2221 GGAGACTCTGGGGATCTGGAGCTGCAGGCAGGAGGTGGCACTGGCTCCCACTCCCACCCC
2281 TGCCCAGGCTGGCATCTAGAAGGCGTCATGAATTACTTTCTCTTCTCTCTTCTCAATTTT
2341 GAGGTCCTCATTCCCAAGATTGAGGAGGCAGTAGTTAATCTGGGAAGGCAGTAGAATGGC
2401 CAGCATTCCTGCCTGTAAGTCTTCCCAAGACAGAGGCCTGGTGACACAGTTCAGCCAGGA
2461 CTGACCACAGGGCTCTAGAGCTCTCTTTGGTGAGACTTCCCTGGATGGAGAGCAGCAGCA
2521 GGGGAAGAGGTGCTCTCAGAGACAGCAGGGCTGGTGCTCTTCTCCCACAAGCTGAGCTCC
2581 ACGTTCAGCAGATACTGTCCAAGGCAGGGGTACGGCTGACCAGGAATGAAGGTTGAACTC
2641 TGCTCCTGAGCACGGTGCGTGCAAAGCATATAGCAGCACATAGGCTCAGGCTTCTGTAGG
2701 CTTCCTGTCCCAGAGCCAATTATGGAAGTAAGGGCTTCCCTCCAGCTAGTCACTGGAATG
2761 GAAAAGTGTGTGTCCTGTTCATAGCCAGGAAACCCAGCTCAGCAAACTCCCTTTCAAAGC
2821 TGTGTGACCGGCTGGGCATGGTGGCTCACACCTGTAATCCCAGCACTTTGGGAGGCCAAG
2881 GCAGGCAATCACCTGAGGTCAGGAGTTCAAGACCAGCCTGGCTAACATGTGAAACTAATA
2941 ATAATACAAAAATTAGCTGGGCGTGGTGGCACATGCCTGTAATCCCAGCTACTTGGGAGG
3001 CTGAGTTGGGAGGATTGCTGCAATCTGGGAGGTGGAAGTTGCAGTGAGCCGAGATCATGC
3061 CACTGCACTCCAGCCTGGGCGACGGAGTGAGACTCCATCTCAAAAAAAAAAAAAATAAAA
3121 ATAAAAGCTGTGTGACCTTGGGCAASCCTGTAGCCTCTCTGGGTCTGTTTCCCTGTCTGG
```

FIG. 6E

DNA and Translated Amino Acid Sequence of TPC3 ORF

```
3181 GTTAAATGGCCTGTAAGGTCCTAGCCAGCTCTACATTCTGCATTTGCTCGCAACCTTGTA
3241 ACACAGAAGTTTTTAGTTAAATTGACAACAGAAGGTTCTCAAAAGCACAATATATGAAGT
3301 AGGAAATTACTATTGCCTTTCTGTGGAGCAAGGGGTGTTGTACACACAAGCCTCACTGTA
3361 GACACTGCCTCAGTTTCCCCATAGGCATAATGGGTCCCTTCTAGTTCAGGCAATCTGGAT
3421 TTGATCTTGAGTTCCAGTGCCAGCCTCTGGAGTCACTCCATTTTCATACCTTTTCATGAT
3481 CTCAGGGGCTCTGGGCAGTGGGAGGTGATGGCTTGGACAGATTCTTGGTCATGCTCCCCA
3541 ACTCTTGGTGGCTCACCACTGAACACTCCAAACCCTGCTTAAAGAAGTTGATTTATTTGA
3601 AAGCCAGGGTAAAGATTGCTAAGGCTTGTCTCCTCTCCCAGTGGGAAGAGAGAGGTTCTG
3661 TTGGTGTCCTGGTTGAATTGCTTTGCAGAGAAGTCAATGCCCATCACCCTTGATGGGGGT
3721 CAGCCTAGGCTGGGGCAGATGGAGAAGGCTTTGGACAGGAAAAAAGTGAGCAGGATGGTA
3781 GTCTAGGCCAGGAGAAGTGTTTGAACAAAGCAGCAGAGATGAGACTCAGTAGACCATGGG
3841 AAGGGGGTGGCTGGCTTCACGAGAGGTGGGGGCTAAGGGGCCTGGAATCCAGGCTAAAGA
3901 CCACACCTACATGTGGCAAGCACCAAGACAGGCATTTGAGGGTTTCCAAATCCTCAGGTC
3961 TCTTGCTGGGGTCTGGAATTTGGAAGGGGAATCCACCAGCCATGGGGGCATCAGAGGAGA
4021 GACTTAGGCAGCGCTGTGGGAGGTTGGCAGATTCCAGGAGTGACAGAGGAGGTTTTTGGT
```

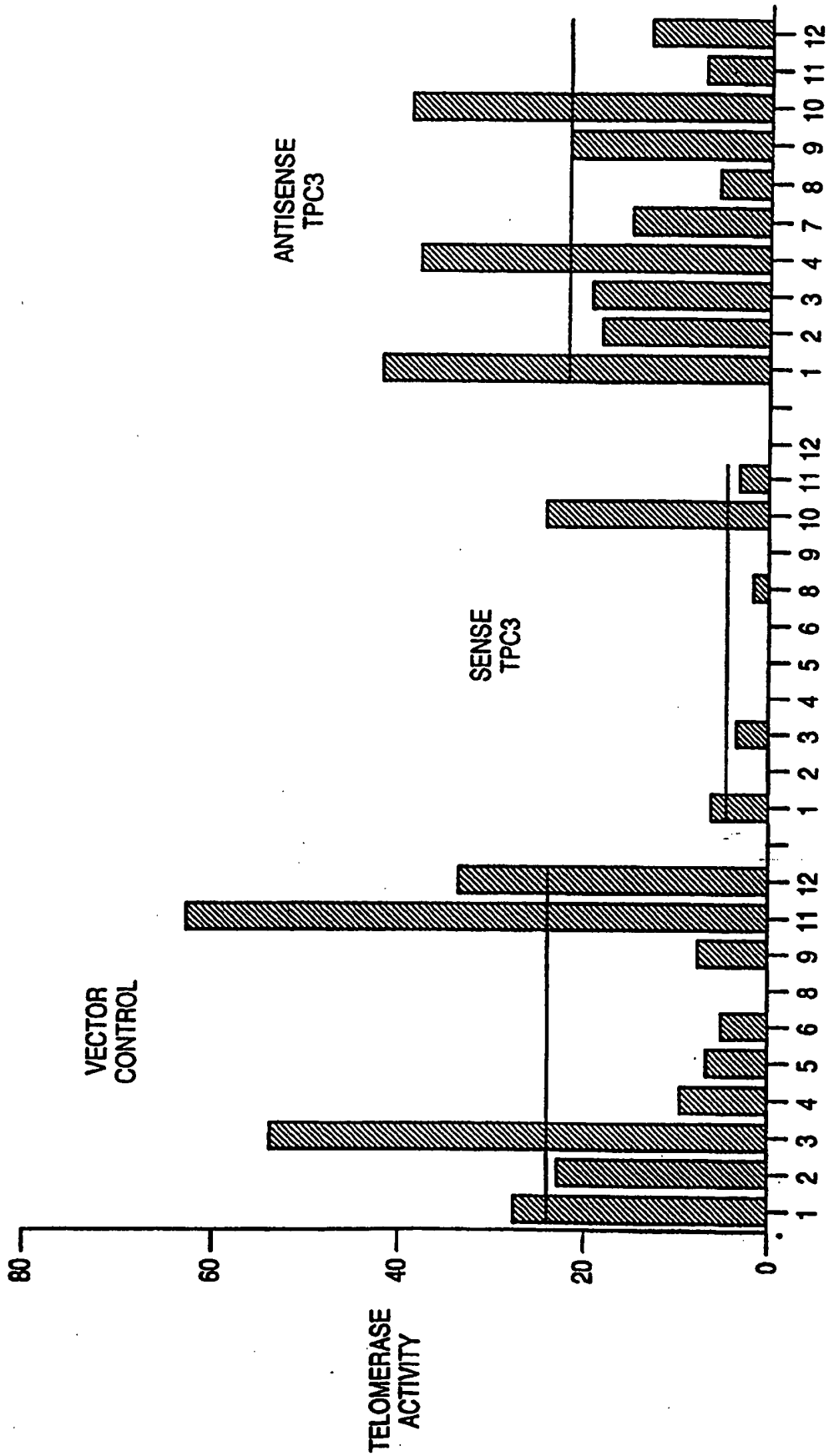

TELOMERASE ACTIVITY
0
20
40
60
80
VECTOR CONTROL
SENSE TPC3
ANTISENSE TPC3
1 2 3 4 5 6 8 9 10 11 12
CLONES

FIG. 7


ANTISENSE TPC3
SENSE TPC3
VECTOR CONTROL
1 2 3 4 6 8 9 11 12
CLONES
0 1 2 3 4 5 6
MEAN TRF

FIG. 8

FIG. 9A

DNA and Sequence of hTR Gene and Transcript

```
   1 CTGCAGAGGATAGAAAAAAG0CCCTCTGATACCTCAAGTTAGTTTCACCTTTAAAGAAGG
     -PsT1-

  61 TCGGAAGTAAAGACGCAAAGCCTTTCCCGGACGTGCGGAAGGGCAACGTCCTTCCTCATG

 121 GCCGGAAATGGAACTTTAATTTCCCGTTCCCCCCAACCAGCCCGCCCGAGAGAGTGACTC

 181 TCACGAGAGCCGCGAGAGTCAGCTTGGCCAATCCGTGCGGTCGGCGGCCGCTCCCTTTAT

                                          ************************************
 241 AAGCCGACTCGCCCGGCAGCGCACCGGGTTGCGGAGGGTGGGCCTGGGAGGGGTGGTGGC

     ************************************************************
 301 CATTTTTTGTCTAACCCTAACTGAGAAGGGCGTAGGCGCCGTGCTTTTGCTCCCCGCGCG

     ************************************************************
 361 CTGTTTTTCTCGCTGACTTTCAGCGGGCGGAAAAGCCTCGGCCTGCCGCCTTCCACCGTT

     ************************************************************
 421 CATTCTAGAGCAAACAAAAAATGTCAGCTGCTGGCCCGTTCGCCCCTCCCGGGGACCTGC
```

FIG. 9B

DNA and Sequence of hTR Gene and Transcript

hTR

```
    **************************************************************
481 GGCGGGTCGCCTGCCCAGCCCCCGAACCCCGCCTGGAGGCCGCGGTCGGCCCGGGGCTTC

    **************************************************************
541 TCCGGAGGCACCCACTGCCACCGCGAAGAGTTGGGCTCTGTCAGCCGCGGGTCTCTCGGG

    **************************************************************
601 GGCGAGGGCGAGGTTCAGGCCTTTCAGGCCGCAGGAAGAGGAACGGAGCGAGTCCCCGCG

    ***************************************************>
661 CGCGGCGCGATTCCCTGAGCTGTGGGACGTGCACCCAGGACTCGGCTCACACATGCAGTT

721 CGCTTTCCTGTTGGTGGGGGGGAACGCCGATCGTGCGCATCCGTCACCCCTCGCCGGCAGT

781 GGGGGCTTGTGAACCCCCAAACCTGACTGACTGGGCCAGTGTGCTGCAAATTGGCAGGAG

841 ACGTGAAGGCACCTCCAAAGTCGGCCAAAATGAATGGGCAGTGAGCCGGGGTTGCCTGGA

901 GCCGTTCCTGCGTGGGTTCTCCCGTCTTCCGCTTTTTGTTGCCTTTTATGGTTGTATTAC

961 AACTTAGTTCCTGCTCTGCAG
                  -PstI-
```

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/14679

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :Please See Extra Sheet.
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/69.1, 69.7, 172.3, 193, 194, 240.1, 240.2, 320.1; 530/300, 358, 387.9, 388.26, 389.1; 536/23.1, 23.2, 23.4, 23.5, 24.31

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	HARA et al. Structure and evolution of four POU domain genes expressed in mouse brain. Proc. Nat. Acad. Sci, USA. April 1992. Vol. 89. pages 3280-3284, especially mouse brain -1 POU domain protein nucleotides 1705-1728.	1-3, 5, 9, 13 --- 7
A	COLLINS. Structure and function of telomerase. Curr. Opion. Cell Biol. 1996. Vol. 8. No. 3. pages 374-380, entire document.	1-20
X --- Y	Databases EST/EST-TWO on MPSEARCH. Accession No. H51230. HILLIER et al. 'The WashU-Merck EST project', sequence yo33b06.r1 Homo sapiens cDNA clone 179699. 18 September 1995.	3, 4 --- 1, 2, 6, 8, 10, 12, 14, 16, 17, 19, 20

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
18 DECEMBER 1996	31 JAN 1997
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer GABRIELE E. BUGAISKY Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/14679

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/14679

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	Databases EST-STS and EST-STS-Two on MP-SEARCH. Accession No. T29492. ADAMS et al. "Initial assessment of human gene diversity and expression patterns based upon 52 million basepairs of cDNA sequence", sequence of EST81752 Homo sapiens cDNA 3' end similar to U1 small nuclear ribonucleoprotein, 70 kDA. 06 September 1995.	3, 5, 9 --- 1, 2, 7, 13
A	DE LANGE. *In* search of vertebrate telomeric proteins. Seminars in Cell & Developmental Biology. February 1996. Vol. 7, No. 1. pages 23-29, entire document.	1-20

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/14679

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (6):

C12N 5/16, 9/12, 15/12, 15/54, 15/62, 15/63; C07K 2/00, 14/47, 16/40, 16/18

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

435/194, 240.1, 320.1; 530/300, 358, 387.9; 536/23.2, 23.4, 23.5, 24.31

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

APS, STN files Registry, CA, Dialog files 155, 5, 434, 351, 301 (Medline, Biosis, Sciscarch, Derwent WPI 1981-1996, Chemname) A-geneseq24, PIR47, Swiss-prot32, EMBL-new3, Genbank92, Genbank-new1, uembl45 92, N-geneseq24, EST-STS, EST-STS-TWO
search terms: telomerase, telomer?, protein, subunit?, component?, gene? ?, nucleotidyltransferase, deoxyribonucleotidyltransferase, ataxia telangiectasia, autoantibodies, autoantisera, antibod?, antiser?

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This application contains claims directed to more than one species of the generic invention. These species are deemed to lack Unity of Invention because they are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for more than one species to be searched, the appropriate additional search fees must be paid. The species are as follows:

1) Nucleic acid encoding TPC2, TPC2 and antibodies thereto and
2) Nucleic acid encoding TPC3, TPC3 and antibodies thereto.

The claims are deemed to correspond to the species listed above in the following manner:

Claims 1-4, 6, 8, 10, 12, 14, 16, 17, 19 and 20 are directed to TPC2.

Claims 1-3, 5, 7, 9, 11, 13, 15, 16 and 18-20 are directed to TPC3.

The following claims are generic: 1-3, 16, 19 and 20.

The species listed above do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, the species lack the same or corresponding special technical features for the following reasons: TPC2 and TPC3 are products of different genes and by their nature do not share the same technical feature. PCT Rules 13.1 and 13.2 do not provide for multiple products.